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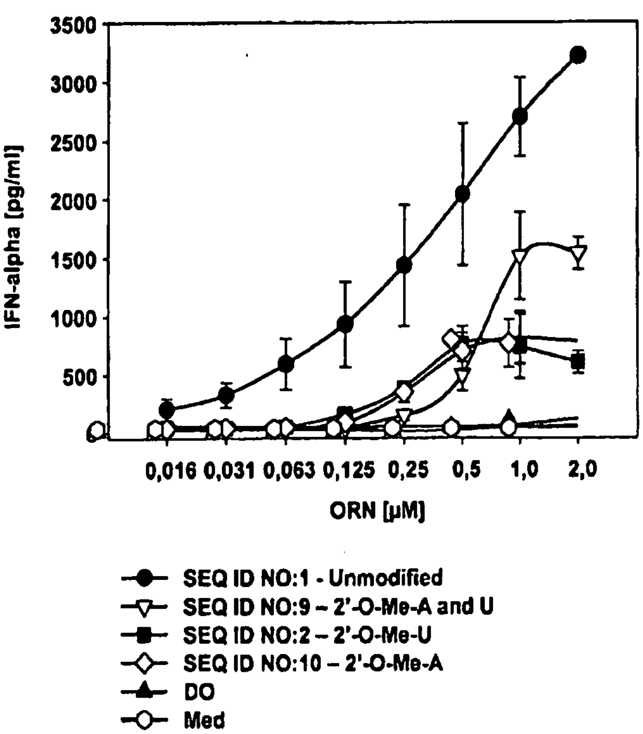
(54) Title: IMMUNE MODULATION BY CHEMICALLY MODIFIED RIBONUCLEOSIDES AND OLIGORIBONUCLEOTIDES



(57) Abstract: The invention relates to modified oligoribonucleotides with immunomodulatory activity. The invention encompasses treatment of autoimmune and infectious diseases using the oligonucleotides of the invention.

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IMMUNE MODULATION BY CHEMICALLY MODIFIED RIBONUCLEOSIDES AND OLIGORIBONUCLEOTIDES

FIELD OF THE INVENTION

The invention relates generally to the field of immunology, and more particularly to immunomodulatory molecules. More specifically the invention relates to modified ribonucleic acid (RNA) molecules, including oligoribonucleotides, with immunosuppressive activity.

BACKGROUND OF THE INVENTION

Toll-like receptors (TLRs) are a family of highly conserved pattern recognition receptor (PRR) polypeptides that recognize pathogen-associated molecular patterns (PAMPs) and play a critical role in innate immunity in mammals. Currently at least ten family members, designated TLR1 - TLR10, have been identified. The cytoplasmic domains of the various TLRs are characterized by a Toll-interleukin 1 receptor (TIR) domain. Medzhitov R et al. (1998) *Mol Cell* 2:253-8. Recognition of microbial invasion by TLRs triggers activation of a signaling cascade that is evolutionarily conserved in *Drosophila* and mammals. The TIR domain-containing adapter protein MyD88 has been reported to associate with TLRs and to recruit interleukin 1 receptor-associated kinase (IRAK) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) to the TLRs. The MyD88-dependent signaling pathway is believed to lead to activation of NF-κB transcription factors and c-Jun NH_2 terminal kinase (Jnk) mitogen-activated protein kinases (MAPKs), critical steps in immune activation and production of inflammatory cytokines. For reviews, see Aderem A et al. (2000) *Nature* 406:782-87, and Akira S et al. (2004) *Nat Rev Immunol* 4:499-511.

A number of specific TLR ligands have been identified. Ligands for TLR2 include peptidoglycan and lipopeptides. Yoshimura A et al. (1999) *J Immunol* 163:1-5; Yoshimura A et al. (1999) *J Immunol* 163:1-5; Aliprantis AO et al. (1999) *Science* 285:736-9. Lipopolysaccharide (LPS) is a ligand for TLR4. Poltorak A et al. (1998) *Science* 282:2085-8; Hoshino K et al. (1999) *J Immunol* 162:3749-52. Bacterial flagellin is a ligand for TLR5. Hayashi F et al. (2001) *Nature* 410:1099-1103. Peptidoglycan has been reported to be a ligand not only for TLR2 but also for TLR6. Ozinsky A et al. (2000) *Proc Natl Acad Sci*

USA 97:13766-71; Takeuchi O et al. (2001) *Int Immunol* 13:933-40. Recently certain low molecular weight synthetic compounds, the imidazoquinolines imiquimod (R-837) and resiquimod (R-848), were reported to be ligands of TLR7 and TLR8. Hemmi H et al. (2002) *Nat Immunol* 3:196-200; Jurk M et al. (2002) *Nat Immunol* 3:499.

Beginning with the recent discovery that unmethylated bacterial DNA and synthetic analogs thereof (CpG DNA) are ligands for TLR9 (Hemmi H et al. (2000) *Nature* 408:740-5; Bauer S et al. (2001) *Proc Natl Acad Sci USA* 98, 9237-42), it has been reported that ligands for certain TLRs include certain nucleic acid molecules. Recently it has been reported that certain types of RNA are immunostimulatory in a sequence-independent or sequence-dependent manner. Further, it has been reported that these various immunostimulatory RNAs stimulate TLR3, TLR7, or TLR8.

SUMMARY OF THE INVENTION

The invention is based in part on the discovery that modifications of specific nucleotides in single stranded oligoribonucleotides (ORN) outside the immune modulatory motif can result in suppression of the immunomodulatory capacity of the ORN. It was discovered that 2'O-methyl modification of rA, rG or rU, but not rC, nucleosides within a stimulatory ORN produced a molecule having reduced immunostimulatory potential compared to unmodified versions of the same ORN. Additionally, certain 2' modified ORN (either single-stranded ORN, whole RNA or 18S rRNA) act as TLR-7, -8, or -9 antagonists by suppressing immune stimulation of ligands.

One aspect of the invention is a method for treating autoimmune disease in a subject, comprising administering to a subject in need of such treatment an effective amount for treating autoimmune disease of a modified oligoribonucleotide having an immune modulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a nucleoside 3' or 5' of the immune modulatory motif. In one embodiment, the modification is within 9 nucleotides of the immune modulatory motif. In another embodiment, the 2' modification of the immune modulatory motif decreases immune stimulatory activity of the ORN containing the motif. In yet another embodiment, the autoimmune disease involves antibody-mediated or T-cell mediated immunity. In some embodiments the autoimmune disease is selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia

areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo. In one embodiment the 2' modification is on a rA, rG or rU residue. In another embodiment the 2' modification is O-methyl. In one embodiment the nucleobase of the modified residue is selected from a group consisting of hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(C1-C6)-alkylcytosine, 5-methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine, 5-bromocytosine, N2-dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4-diaminopurine, 2,6-diaminopurine, 8-azapurine, substituted 7-deazapurine, 7-deaza-7-substituted purine, 7-deaza-8-substituted purine, hydrogen (abasic residue). In another embodiment the immune modulatory motif has a base sequence selected from 5' U U G U 3', 5' C/U U G/U U 3', 5' R U R G Y 3', 5' G U U G B 3', 5' G U G U G/U 3', 5' G/C U A/C G G C A C 3', and N-U-R1-R2, where C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and wherein at least one of R1 and R2 is adenosine (A) or cytosine (C) or derivatives thereof and wherein R is not U unless N-U-R1-

R2 includes at least two A. In one embodiment the subject is a subject having autoimmune disease. In another embodiment the subject is a subject at risk of developing autoimmune disease. In yet another embodiment, the modified oligoribonucleotide is single stranded and the oligoribonucleotide sequence is not complementary to a coding sequence in the target cell. In one embodiment the modified oligoribonucleotide comprises at least one 2' modified nucleoside in the immune modulatory motif.

Another aspect of the invention is a method for treating an inflammatory disorder in a subject, comprising administering to a subject in need of such treatment an effective amount for treating an inflammatory disorder of a modified oligoribonucleotide having an immune modulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a nucleoside 3' or 5'of the immune modulatory motif. In one embodiment the subject is a subject at risk of developing an inflammatory disorder. In one embodiment the inflammatory disorder is sepsis. In another embodiment the inflammatory disorder is an infection. In one embodiment the 2' modification is on a rA, rG or rU residue. In another embodiment the 2' modification is O-methyl. In yet another embodiment the nucleobase of the modified residue is selected from a group consisting of hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2 thiouracil, 4 thiouracil, 5 aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5 (hydroxymethyl)uracil, 5 chlorouracil, 5 fluorouracil, 5 bromouracil, 5 hydroxycytosine, 5-(C1-C6)-alkylcytosine, 5 methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5 chlorocytosine, 5 fluorocytosine, 5 bromocytosine, N2 dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4 diaminopurine, 2,6-diaminopurine, 8 azapurine, substituted 7 deazapurine, 7 deaza 7 substituted purine, 7 deaza 8 substituted purine, hydrogen (abasic residue). In another embodiment the immune modulatory motif has a base sequence selected from 5' U U G U 3', 5' C/U U G/U U 3', 5' R U R G Y 3', 5' G U U G B 3', 5' G U G U G/U 3', 5' G/C U A/C G G C A C 3', and N-U-R1-R2, where C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and where at least one of R1 and R2 is adenosine (A) or cytosine (C) or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A. In one embodiment the

modified oligoribonucleotide comprises at least one 2' modified nucleoside in the immune modulatory motif.

Another aspect of the invention is a composition comprising a modified oligoribonucleotide, wherein the modified oligoribonucleotide contains at least one 2'modification on a residue 3' or 5'of an immune modulatory motif, wherein the 2' modification is on a rA, rG or rU residue. In one embodiment the 2' modification is an O-methyl. In another embodiment the nucleobase of the modified residue is selected from a group consisting of hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2 thiouracil, 4 thiouracil, 5 aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5 (hydroxymethyl)uracil, 5 chlorouracil, 5 fluorouracil, 5 bromouracil, 5 hydroxycytosine, 5-(C1-C6)-alkylcytosine, 5 methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5 chlorocytosine, 5 fluorocytosine, 5 bromocytosine, N2 dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4 diaminopurine, 2,6-diaminopurine, 8 azapurine, substituted 7 deazapurine, 7 deaza 7 substituted purine, 7 deaza 8 substituted purine, hydrogen (abasic residue). In one embodiment the modified oligoribonucleotide has a backbone modification. In one embodiment the backbone modification is a phosphorothioate modification. In another embodiment the modified oligoribonucleotide is between 10 and 30 nucleotides in length. In yet another embodiment the modified oligoribonucleotide contains at least two modified residues. In still another embodiment the modified oligoribonucleotide contains at least three modified residues. In another embodiment the immune modulatory motif has a base sequence selected from 5' U U G U 3', 5' C/U U G/U U 3', 5' R U R G Y 3', 5' G U U G B 3', 5' G U G U G/U 3', 5' G/C U A/C G G C A C 3', and N-U-R1-R2, where C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and where at least one of R1 and R2 is Adenosine (A) or Cytosine or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A. In another embodiment the modified oligoribonucleotide is single stranded and wherein the oligoribonucleotide sequence is not complementary to a coding sequence in the target cell. In one embodiment the modified

oligoribonucleotide comprises at least one 2' modified nucleoside in the immune modulatory motif

Another aspect of the invention is a method for suppressing an immune response in a subject by administering to a subject in need of such treatment any one of the modified oligoribonucleotides of the invention. In one embodiment, the immune response is an RNA-mediated immune response. In another embodiment the immune response is a DNA-mediated immune response. In one embodiment, the subject has an autoimmune disease. In another embodiment the subject is at risk of developing an autoimmune disease. In another embodiment the subject has an inflammatory disorder. In one embodiment the suppression of the immune response comprises suppression of TLR8 signaling. In another embodiment the suppression of the immune response comprises suppression of TLR7 signaling. In still another embodiment the suppression of the immune response comprises suppression of TLR9 signaling. In another embodiment the suppression of the immune response comprises suppression of activation of antigen-presenting cells, B cells, myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), monocytes, monocyte-derived cells, eosinophils, or neutrophils. In one embodiment the subject is administered a TLR ligand. In another embodiment the TLR ligand is a CpG oligonucleotide. In yet another embodiment the ligand is an immune stimulatory RNA. In still another embodiment the modified oligoribonucleotide is single stranded and wherein the oligoribonucleotide sequence is not complementary to a coding sequence in the target cell. In another embodiment the ligand is a small molecule.

Another aspect of the invention is a method of inhibiting an RNA-mediated immune response in a subject, comprising administering to a subject in need of such treatment a 2'-modified cytidine. In one embodiment the 2'-modified cytidine is 2'-O-methyl cytidine. In another embodiment the 2'-modified cytidine is 2'-O-alkyl cytidine. In one embodiment, the 2'-O-alkyl-modification is 2'-O-ethyl, 2'-O-propyl or 2'-O-butyl. In another embodiment, the 2'-modified nucleoside is a 2'-O, 4'-C-alkylen-bridged nucleoside, e.g. 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine. In one embodiment, the 2'-O-alkyl-modification contains at least one unsaturated carbon-carbon linkage, e.g. 2'-O-allyl or 2'-O-propinyl. In one embodiment, the 2'-O-alkyl-modification is 2'-O-(2-methoxyethyl). In one embodiment the subject is a subject having or at risk of having a condition selected from the group comprising scleroderma, juvenile rheumatoid arthritis,

ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

Another aspect of the invention is a method of inhibiting a DNA-mediated immune response in a subject, comprising administering to a subject in need of such treatment a 2'-modified cytidine. In one embodiment the 2'-modified cytidine is 2'-O-methyl cytidine. In another embodiment the 2'-modified cytidine is 2'-O-alkyl cytidine. In one embodiment, the 2'-O-alkyl-modification is 2'-O-ethyl, 2'-O-propyl or 2'-O-butyl. In another embodiment, the 2'-modified nucleoside is a 2'-O, 4'-C-alkylen-bridged nucleoside, e.g. 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine. In one embodiment, the 2'-O-alkyl-modification contains at least one unsaturated carbon-carbon linkage, e.g. 2'-O-allyl or 2'-O-propinyl. In one embodiment, the 2'-O-alkyl-modification is 2'-O-(2-methoxyethyl). In one embodiment the subject is a subject having or at risk of having a condition selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed

cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

Another aspect of the invention is a method of treating an autoimmune response in a subject, comprising administering to a subject in need of such treatment a 2'-modified cytidine. In one embodiment the 2'-modified cytidine is 2'-O-methyl cytidine. In another embodiment the 2'-modified cytidine is 2'-O-alkyl cytidine. In one embodiment, the 2'-O-alkyl-modification is 2'-O-ethyl, 2'-O-propyl or 2'-O-butyl. In another embodiment, the 2'-modified nucleoside is a 2'-O, 4'-C-alkylen-bridged nucleoside, e.g. 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine. In one embodiment, the 2'-O-alkyl-modification contains at least one unsaturated carbon-carbon linkage, e.g. 2'-O-allyl or 2'-O-propinyl. In one embodiment, the 2'-O-alkyl-modification is 2'-O-(2-methoxyethyl). In yet another embodiment the subject is a subject having or at risk of having a condition selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's

phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

Another aspect of the invention is a method for suppressing an immune response in a subject by administering to a subject in need of such treatment any one of the 2'-modified cytidines of the invention. In one embodiment, the immune response is a RNA-mediated immune response. In another embodiment, the immune response is a DNA-mediated immune response. In one embodiment, the subject has an autoimmune disease. In another embodiment the subject is at risk of developing an autoimmune disease. In another embodiment the subject has an inflammatory disorder. In one embodiment the suppression of the immune response comprises suppression of TLR8 signaling. In another embodiment the suppression of the immune response comprises suppression of TLR7 signaling. In still another embodiment the suppression of the immune response comprises suppression of TLR9 signaling. In another embodiment the suppression of the immune response comprises suppression of activation of antigen-presenting cells, B cells, myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), monocytes, monocyte-derived cells, eosinophils, or neutrophils. In one embodiment the subject is administered a TLR ligand. In another embodiment the TLR ligand is a CpG oligonucleotide. In yet another embodiment the ligand is an immune stimulatory RNA.

Another aspect of the invention is a method for stimulating an immune response, comprising administering to a subject an effective amount for stimulating an immune response in the subject of a modified oligoribonucleotide having an immune stimulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a rC residue 3' or 5'of the immune stimulatory motif. In one embodiment, the immune stimulatory motif has a base sequence selected from 5' U U G U 3', 5' C/U U G/U U 3', 5' R U R G Y 3', 5' G U U G B 3', 5' G U G U G/U 3', 5' G/C U A/C G G C A C 3', and N-U-R1-R2, where C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and where at least one of R1 and R2 is adenosine (A) or cytosine (C) or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A. In one embodiment the the modified oligoribonucleotide comprises at least one 2' modified nucleoside in the immune modulatory motif.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is two graphs demonstrating that 2'-O-Me modification interferes with the stimulatory effect induced by an oligoribonucleotide. Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. Supernatants (SN) were collected and cytokines measured by ELISA. The assay compares an unmodified ORN (SEQ ID NO:1), a 2'-O-Me-A and U modified ORN (SEQ ID NO:9), a 2'-O-Me-U modified ORN)SEQ ID NO:2) and a 2'-O-Me-A modified ORN SEQ ID NO:10) which all show induced induction of IFN-α (FIG. 1a) and TNF-α (FIG. 1b). The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 2 is two graphs demonstrating that 2'-O-Me modification inside the GU stimulatory RNA motif interferes with the oligoribonucleotide stimulatory effect. Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. SN were collected and cytokines measured by ELISA. The assay compares an unmodified ORN (SEQ ID NO:15), FIG. 2a shows IFN-α concentration and FIG. 2b shows TNF-α concentration. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 3 is two graphs demonstrating that 2'-O-Me modification outside of the GU stimulatory RNA motif interferes with the oligoribonucleotide stimulatory effect. Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. SN were collected and cytokines measured by ELISA. FIG. 3a shows IFN-α concentration and FIG. 3b shows TNF-α concentration. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 4 is two graphs demonstrating that 2'-O-Me modification of rU, rG and rA, but not rC interferes with RNA-mediated immune effects. Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. SN were collected and cytokines measured by ELISA. FIG. 4a shows IFN-α concentration and FIG. 4b shows TNF-α concentration. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 5 is two graphs demonstrating that the lack of suppressive effect of the 2'-O-Me modification of rC is position-independent. Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. SN were collected and cytokines measured by ELISA. FIG. 5a shows IFN-α concentration and FIG. 5b shows TNF-α concentration. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 6 is four graphs demonstrating that 2'-O-Me modified oligoribonucleotide can act as antagonist for the immune response (IFN-α and TNF-α) induced by a stimulatory oligoribonucleotide. (FIG.6a and 6c) Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. SN were collected and cytokines measured by ELISA. (FIG. 6b and 6d) Human PBMC of three healthy blood donors were incubated for 24h with 1μM ORN SEQ ID NO:1 in the presence of DOTAP, and were co-cultured with different doses of the indicated compounds (modified ORN SEQ ID NO:2, S-Class ODN SEQ ID NO:3, chloroquine). SN were collected and cytokines measured by ELISA. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 7 is four graphs demonstrating that 2'-O-Me modified oligoribonucleotide can act as antagonist for the immune response (IL-12 and IFN-γ) induced by a stimulatory oligoribonucleotide. (FIG.7a and 7c) Human PBMC of three healthy blood donors were incubated for 24h with the indicated amounts of unmodified or modified oligoribonucleotide in the presence of DOTAP. SN were collected and cytokines measured by ELISA. (FIG. 7b and 7d) Human PBMC of three healthy blood donors were incubated for 24h with 1μM ORN SEQ ID NO:1 in the presence of DOTAP, and were co-cultured with different doses of the indicated compounds (modified ORN SEQ ID NO:2, S-Class ODN SEQ ID NO:3, chloroquine). SN were collected and cytokines measured by ELISA. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 8 is two graphs demonstrating that phosphorothioate 2'-modified oligoribonucleotide suppress oligoribonucleotide -mediated effects stronger than phosphodiester modified oligoribonucleotide, although suppressive phosphorothioate 2'-modified oligoribonucleotide act as suppressors of the response to both, phosphodiester and phosphorothioate RNA. Human PBMC of three healthy blood donors were incubated for 24h with 0.25μM ORN SEQ ID NO:7 (FIG. 8a, phosphorothioate) or SEQ ID NO:5 (FIG. 8b,

phosphodiester) in the presence of DOTAP, and were co-cultured with different doses of the indicated compounds (phosphorothioate 2'-modified ORN SEQ ID NO:2 or phosphodiester 2'-modified ORN SEQ ID NO:6). SN were collected and cytokines measured by ELISA. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 9 is two graphs demonstrating that the suppressive effect depends on the presence of a 2' modified nucleotide. Human PBMC of three healthy blood donors were incubated for 24h with 0.25μM ORN SEQ ID NO:7 in the presence of DOTAP, and were co-cultured with different doses of the indicated compounds (phosphorothioate unmodified ORN SEQ ID NO:16 or 2'-modified ORN SEQ ID NO:8 with the same sequence, or SEQ ID NO:2). SN were collected and cytokines measured by ELISA. FIG. 9a shows IFN-α concentration and FIG. 9b shows TNF-α concentration. The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 10 is a graph demonstrating that CpG ODN-mediated cytokine induction is inhibited by 2'-modified oligoribonucleotide. Human PBMC of three healthy blood donors were incubated for 24h with 0.25μM of the C-Class CpG ODN SEQ ID NO:4 in the presence of DOTAP, and were co-cultured with different doses of the indicated compounds (unmodified ORN SEQ ID NO:16 or 2'-modified ORN SEQ ID NO:2). SN were collected and cytokines measured by ELISA. The x-axis is ORN concentration in μM and the y-axis is cytokine IFN-α in pg/ml.

FIG. 11 is four graphs demonstrating that RNA-mediated immune effects can be inhibited by single 2' modified nucleosides, especially 2'-O-Me-C, and also 2'-O-Me-A. Human PBMC of three healthy blood donors were incubated for 24h with 1μM ORN SEQ ID NO:1 in the presence of DOTAP, and were co-cultured with different doses of the indicated nucleosides, S-Class ODN SEQ ID NO:3 or chloroquine (CQ). SN were collected and cytokines measured by ELISA; IFN-α (FIG. 9a), IFN-γ (FIG. 9b), TNF-α (FIG. 9c) and IL-12p40 (FIG. 9d). The x-axes are ORN concentration in μM and the y-axes are cytokine concentration in pg/ml.

FIG. 12 is a graph demonstrating that single 2' modified nucleosides do not exert an effect on LPS-mediated TNF-α induction with the exception of 2'-O-Me-A. Human PBMC of three healthy blood donors were incubated for 24h with 100ng/ml LPS, and were co-cultured with different doses of the indicated nucleosides. SN were collected and cytokine

(TNF-α) concentration measured by ELISA. The x-axis is ORN concentration in μM and the y-axis is TNF-α concentration in pg/ml.

Fig. 13 is a graph demonstrating that DNA-mediated immune effects can be inhibited by single 2' modified nucleosides, especially 2'-O-Me-C. Human PBMC of three healthy blood donors were incubated for 24h with 0.25μM of the C-Class CpG ODN SEQ ID NO:4, and were co-cultured with different doses of the indicated nucleosides or chloroquine. SN were collected and cytokines measured by ELISA. The x-axis is ORN concentration in μM and the y-axis is IFN-α concentration in pg/ml.

Fig. 14 is three graphs demonstrating that 2'-O-Me-C mediates its strongest suppressive effect on CpG-mediated responses, not all RNA-dependent effects are affected by the 2'-modified nucleoside. Human PBMC of three healthy blood donors were incubated for 24h either with 0.25μM of the C-Class CpG ODN SEQ ID NO:4, 100ng/ml LPS, or 1μM ORN SEQ ID NO:1 (the latter in the presence of DOTAP). PBMC were co-cultured with the indicated doses of 2'-O-Me-C, S-Class ODN SEQ ID NO:3 or chloroquine. SN were collected and cytokines and chemokines measured by Luminex cytokine array. The x-axes represent % inhibition of the indicated ORN.

Figure 15 is a graph showing the effect of 2'-O-Methyl modified ORN on murine TLR7-induced activation of cytokine induction. RAW264 murine macrophages were stimulated for 20h with 1.0μM (splenocytes) or 0.25μM (RAW264) ORN SEQ ID NO:7 complexed to DOTAP, or in the presence of the indicated concentrations of the unmodified non-stimulatory ORN SEQ ID NO:16, or the same sequence with a single 2'-O-methyl modification (SEQ ID NO:8), and TNF-α concentration measured. The x asix is ORN concentration in μM and the y-axis is TNF-α concentration in pg/ml.

Figure 16 is two graphs showing the suppressive effect of 2'-O-Methyl modified ORN on on murine TLR7-induced activation of cytokine induction. Murine splenocytes were treated with stimulatory ORN SEQ ID NO:7 along with either the unmodified non-stimulatory ORN SEQ ID NO:16, or the same sequence with a single 2'-O-methyl modification (SEQ ID NO:8) at the concentrations indicated, and concentrations of IL-12 (Figure 16a) and Il-6 (Figure 16b) in the supernatants were measured. The x-axes are ORN used and the y-axes are cytokine concentration in pg/ml.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based in part on the discovery by the applicants that certain modifications of oligoribonucleotides (ORN) can reduce their immune stimulatory capacity. Immune stimulatory oligonucleotides are known to activate signaling by any one of or any combination of toll receptors known to activate immune cells, including toll-like receptors (TLRs) 7, 8, and 9. Thus, modifications that reduce the immune stimulatory capacity of ORN can effectively modulate their effect on the immune system. Additionally, it was discovered that such modified ORN, as well as specific 2' modified nucleosides, have the ability to suppress the immune stimulatory capacity of TLR ligands, and thus to function as TLR antagonists.

Toll-like receptors (TLRs) are a family of highly conserved polypeptides that play a critical role in innate immunity in mammals. Currently ten human family members, designated TLR1 - TLR10, have been identified. The cytoplasmic domains of the various TLRs are characterized by a Toll-interleukin 1 (IL-1) receptor (TIR) domain. Medzhitov R et al. (1998) *Mol Cell* 2:253-8. Recognition of microbial invasion by TLRs triggers activation of a signaling cascade that is evolutionarily conserved in *Drosophila* and mammals. The TIR domain-containing adapter protein MyD88 has been reported to associate with many of the TLRs and to recruit IL-1 receptor-associated kinase (IRAK) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) to the TLRs. The MyD88-dependent signaling pathway is believed to lead to activation of NF-κB transcription factors and c-Jun NH_2 terminal kinase (Jnk) mitogen-activated protein kinases (MAPKs), critical steps in immune activation and production of inflammatory cytokines. For a review, see Aderem A et al. (2000) *Nature* 406:782-87.

While a number of specific TLR ligands have been reported, ligands for some TLRs remain to be identified. Ligands for TLR2 include peptidoglycan and lipopeptides. Yoshimura A et al. (1999) *J Immunol* 163:1-5; Yoshimura A et al. (1999) *J Immunol* 163:1-5; Aliprantis AO et al. (1999) *Science* 285:736-9. Viral-derived double-stranded RNA (dsRNA) and poly I:C, a synthetic analog of dsRNA, have been reported to be ligands of TLR3. Alexopoulou L et al. (2001) *Nature* 413:732-8. Lipopolysaccharide (LPS) is a ligand for TLR4. Poltorak A et al. (1998) *Science* 282:2085-8; Hoshino K et al. (1999) *J Immunol* 162:3749-52. Bacterial flagellin is a ligand for TLR5. Hayashi F et al. (2001) *Nature* 410:1099-1103. Peptidoglycan has been reported to be a ligand not only for TLR2 but also for TLR6. Ozinsky A et al. (2000) *Proc Natl Acad Sci USA* 97:13766-71; Takeuchi O et al.

(2001) *Int Immunol* 13:933-40. Single-stranded RNA containing guanosine and uridine has been reported to be a ligand for TLR7 and TLR8. U.S. Pat. Appl. Pub. 2003/0232074 A1. Certain low molecular weight synthetic compounds, the imidazoquinolones imiquimod (R-837) and resiquimod (R-848), have also been reported to be ligands of TLR7 and TLR8. Jurk M et al. (2002) *Nat Immunol* 3:499; Hemmi H et al. (2002) *Nat Immunol* 3:196-200. Bacterial DNA (CpG DNA) has been reported to be a TLR9 ligand. Hemmi H et al. (2000) *Nature* 408:740-5; Bauer S et al. (2001) *Proc Natl Acad Sci USA* 98, 9237-42.

It has recently been reported that sequence-nonspecific double-stranded RNA can induce immunostimulatory effects, acting through Toll-like receptor 3 (TLR3). Alexopoulou L et al. (2001) *Nature* 413:732-8. Further, it has also been recently reported that certain single-stranded RNAs, generally including guanosine (G) and uridine (U), and particularly including certain sequence motifs, are also immunostimulatory. Lipford et al. US 2003/0232074 A1. Immunostimulatory single-stranded RNA have been reported to act through Toll-like receptor 7 (TLR7) and Toll-like receptor 8 (TLR8).

In addition to having diverse ligands, the various TLRs are believed to be differentially expressed in various tissues and on various types of immune cells. For example, human TLR7 has been reported to be expressed in placenta, lung, spleen, lymph nodes, tonsil and on plasmacytoid dendritic cells (pDCs). Chuang T-H et al. (2000) *Eur Cytokine Netw* 11:372-8); Kadowaki N et al. (2001) *J Exp Med* 194:863-9. Human TLR8 has been reported to be expressed in lung, peripheral blood leukocytes (PBL), placenta, spleen, lymph nodes, and on monocytes. Kadowaki N et al. (2001) *J Exp Med* 194:863-9; Chuang T-H et al. (2000) *Eur Cytokine Netw* 11:372-8. Human TLR9 is reportedly expressed in spleen, lymph nodes, bone marrow, PBL, and on pDCs, and B cells. Kadowaki N et al. (2001) *J Exp Med* 194:863-9; Bauer S et al. (2001) *Proc Natl Acad Sci USA* 98:9237-42; Chuang T-H et al. (2000) *Eur Cytokine Netw* 11:372-8.

An "immune response" as used herein refers to the response of the immune system to a stimulus. The immune response is conceptually divided into T-cell mediated and antibody mediated immunity. "T-cell mediated immunity" involves recognition of pathogen-associated molecular patterns (PAMPs) shared in common by certain classes of molecules expressed by infectious microorganisms or foreign macromolecules. PAMPs are believed to be recognized by pattern recognition receptors (PRRs) on or in certain immune cells. "Antibody-mediated immunity" involves immune cell activation to produce cytokines that

stimulate B cell antibody synthesis. An immune response may involve activation of B cells, monocytoid dendritic cells, plasmacytoid dendritic cells, monocytes, monocyte-derived cells, and eosinophils, for example. An aberrant immune response, such as one involving excessive or chronic activation of immune cells, can result in detrimental conditions such as autoimmune disease and chronic inflammatory disorders. As used herein, "suppression of activation" refers to the administration of a molecule according to the invention such that activation of immune cells is reduced or eliminated. As used herein, the term "suppressing an immune response" refers to the administration of a molecule according to the methods of the invention such that an immune response is reduced or eliminated. As used herein, an "RNA-mediated immune response" is an immune response activated by an immune stimulatory RNA. Similarly, a "DNA-mediated immune response" is an immune response activated by immune stimulatory DNA.

Certain RNA molecules that are known to have immune stimulatory effects contain a sequence motif thought to be responsible for the immune modulatory activity of the RNA. For example, a base sequence thought to activate TLR8 typically includes at least one guanosine (G) and at least one uracil (U). As used herein, an "immune modulatory motif" is a sequence motif which confers immune modulatory activity to the molecule. The immune modulatory motif in some cases is between 4 and 8 bases long. Lipford et al. US 2003/0232074. Nucleic acid molecules containing GUU, GUG, GGU, GGG, UGG, UGU, UUG, UUU, multiples and any combinations thereof are believed to be TLR8 ligands. RNA molecules may have multiple immune modulatory motifs. As used herein, the term "immune modulatory motif" describes a sequence motif in a molecule that provides the immune modulatory activity to the molecule.

It was surprisingly discovered by the inventors that the immune modulatory motif itself need not be modified to produce an ORN with altered immune modulatory capacity. Certain modifications outside this immune modulatory motif or in ORN with no immune modulatory motif, particularly modifications of A, U, or G, can also reduce the immune modulatory capacity of the ORN. A further surprising aspect of the invention is based on the discovery by the inventors that these modified ORN can exert an immunosuppressive effect alone or in the presence of other immune stimulatory molecules. Another suprising aspect was that 2' modification of an ORN not containing an immune stimulatory motif can exert the immunosuppressive effect. As used herein, the term "modified ORN" refers to an ORN

which includes a residue having a modification at the 2' position on a residue outside the immune modulatory motif.

The modified ORN of the invention may be modified such that one β-ribose unit may be replaced by a modified sugar unit. As used herein, a "2' modification" on an ORN is one in which the ribose on a residue of the ORN has been modified at the 2' position. The modified sugar unit is for example selected from β-D-ribose, α-D-ribose, β-L-ribose (as in 'Spiegelmers'), α-L-ribose, 2'-amino-2'-deoxyribose, 2'-fluoro-2'-deoxyribose, 2'-O-(C1-C6)alkyl-ribose, 2'-O-(C1-C6)alkyl-ribose, 2'-O-methylribose, 2'-O-(C2-C6)alkenyl-ribose, 2'-[O-(C1-C6)alkyl-O-(C1-C6)alkyl]-ribose, LNA and α-LNA (Nielsen P et al. (2002) *Chemistry-A European Journal* 8:712-22), β-D-xylo-furanose, α-arabinofuranose, 2'-fluoro arabinofuranose, and carbocyclic and/or open-chain sugar analogs (described, for example, in Vandendriessche et al. (1993) *Tetrahedron* 49:7223) and/or bicyclosugar analogs (described, for example, in Tarkov M et al. (1993) *Helv Chim Acta* 76:481). As used herein, a "2'-O methyl" modification refers to a modified sugar unit with an O-methyl group at the 2' position.

The suppressive effect of nucleotide modifications in a stimulatory ORN can be attributed to modification of specific ribonucleosides. It was found by the inventors that modification of ribose at the 2' position outside the modulatory motif of the ORN causes a decrease in its immune modulatory activity. As used herein, a "decrease in immune modulatory activity" refers to a reduction or elimination of the ability of the molecule to stimulate an immune response as compared to the same molecule without the modification. The term "stimulate an immune response" refers to any increase in immune parameter, such as, for example, activation of a B or T cell or other immune cell or induction in one or more cytokine levels. This modification has been shown to be effective for reducing immunomodulatory capacity of the ORN when the modification occurs on rA, rG, or rU, as measured by a suppression of production of cytokines (IFN-α, TNF-α, and IFN-γ; see Examples). The 2' modification not only results in suppression of immune modulatory effects of the modified ORN, but has also been found to suppress the immune stimulatory effects of a TLR ligand when added as an inhibitory or antagonistic ORN. In addition, the 2' modification has been found to suppress DNA-mediated effects when added as an inhibitory or antagonistic ORN to stimulatory CpG ODN molecules.

In some embodiments the modified ORN of the invention comprise 2 or more modified residues. In some cases the modified ORN of the invention comprise 3-10 modified residues. According to the methods of the invention, the modified ORN are not designed to comprise a sequence complementary to that of a coding sequence in a human cell, and are therefore not considered to be antisense ORN or silencing RNA (siRNA). An ORN which is "not complementary" is one that does not comprise a sequence capable of hybridizing strongly with one particular coding region in the target cell. Therefore, administration of an ORN which is not complementary as used herein will not result in gene silencing, especially as the ORN described in this invention are single-stranded compared to the double-stranded molecules used as silencing RNAs. The oligonucleotide may have other carbohydrate backbone modifications and replacements, such as peptide nucleic acids with phosphate groups (PHONA), locked nucleic acids (LNA), and oligonucleotides having backbone sections with alkyl linkers or amino linkers. The alkyl linker may be branched or unbranched, substituted or unsubstituted, and chirally pure or a racemic mixture. As used herein, the term "phosphorothioate backbone" refers to a stabilized sugar phosphate backbone of a nucleic acid molecule in which a non-bridging phosphate oxygen is replaced by sulfur at least one internucleotide linkage. In one embodiment non-bridging phosphate oxygen is replaced by sulfur at each and every internucleotide linkage.

A β-ribose unit or a β-D-2'-deoxyribose unit can be replaced by a modified sugar unit, wherein the modified sugar unit is for example selected from β-D-ribose, α-D-2'-deoxyribose, L-2'-deoxyribose, 2'-F-2'-deoxyribose, 2'-F-arabinose, 2'-O-(C_1-C_6)alkyl-ribose, 2'-O-methylribose, 2'-O-(C_2-C_6)alkenyl-ribose, 2'-[O-(C_1-C_6)alkyl-O-(C_1-C_6)alkyl]-ribose, 2'-fNH_2-2'-deoxyribose, β-D-xylo-furanose, α-arabinofuranose, 2,4-dideoxy-β-D-erythro-hexo-pyranose, and carbocyclic (described, for example, in Froehler (1992) *J Am Chem Soc* 114:8320) and/or open-chain sugar analogs (described, for example, in Vandendriessche et al. (1993) *Tetrahedron* 49:7223) and/or bicyclosugar analogs (described, for example, in Tarkov M et al. (1993) *Helv Chim Acta* 76:481). In some embodiments the sugar is 2'-O-methylribose.

In some embodiments the modified ORN of the invention is between 10 and 30 nucleotides in length. In some embodiments the modified ORN is between 10 and 50 nucleotides in length. In some embodiments the modified ORN of the invention is between 10 and 100 nucleotides in length. In some embodiments the modified ORN have a backbone

that may be stabilized. In one embodiment the backbone is a sugar phosphate backbone that includes at least one phosphorothioate internucleotide linkage. In one embodiment the backbone is completely phosphorothioate.

In one aspect the invention provides a method for treating a condition associated with aberrant immune stimulation in a subject. As used herein, the term "treat" as used in reference to a disease or condition shall mean to intervene in such disease or condition so as to prevent or slow the development of, prevent or slow the progression of, halt the progression of, or eliminate the disease or condition. The method according to this aspect of the invention involves the step of administering to a subject having or at risk of developing a condition associated with aberrant immune stimulation an effective amount of an isolated immune modulatory ORN of the invention to treat the condition. The ORN can be but need not be limited to a single administration. The method is useful whenever it is desirable to slow or alter an immune response. For instance, in some cases it is useful to skew an immune response away from a Th1-like immune response. According to this aspect of the invention, immune modulatory ORN of the invention may be used to treat any of a number of conditions that involve an innate immune response or a Th1-like immune response, including inflammation, acute and chronic allograft rejection, graft-versus-host disease (GvHD), certain autoimmune diseases, infection, and sepsis.

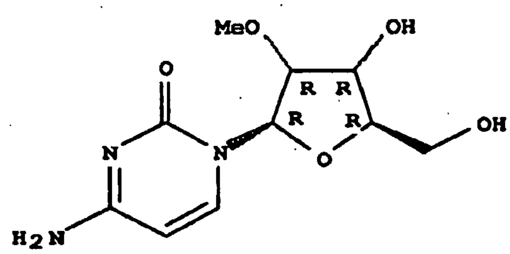
As used herein, the term "subject" refers to a human or non-human vertebrate. Non-human vertebrates include livestock animals, companion animals, and laboratory animals, such as, for instance, non-human primates, chickens, horses, cows, pigs, goats, dogs, cats, guinea pigs, hamsters, mink, and rabbits. As used herein, the term "subject at risk of developing" a condition refers to a subject with a known or suspected exposure to an agent known to cause or to be associated with the condition or a known or suspected predisposition to develop the condition (e.g., a genetic marker for or a family history of the condition).

As used herein "infection" refers to a condition associated with the activation of the immune system by a microorganism, including but not limited to bacteria, fungi, and viruses. As used herein, the term "sepsis" refers to a well-recognized clinical syndrome associated with a host's systemic inflammatory response to microbial invasion. The term "sepsis" as used herein refers to a condition that is typically signaled by fever or hypothermia, tachycardia, and tachypnea, and in severe instances can progress to hypotension, organ dysfunction, and even death. As used herein, the term "autoimmune disease" refers to a

disease caused by a breakdown of self-tolerance such that the adaptive immune system responds to self antigens and mediates cell and tissue damage. An "autoimmune response" is therefore defined as an aberrant immune response resulting in an autoimmune condition or disease. Autoimmune diseases specifically include, without limitation, insulin-dependent diabetes mellitus, inflammatory bowel disease, and multiple sclerosis. Additional specific examples of autoimmune diseases are provided below. Such conditions typically involve activation toll receptor signaling in response to the RNA or DNA.

As used herein, the term "inflammatory disorder" refers to a condition associated with an antigen-nonspecific reaction of the innate immune system that involves accumulation and activation of leukocytes and plasma proteins at a site of infection, toxin exposure, or cell injury. Cytokines that are characteristic of inflammation include tumor necrosis factor (TNF-α), interleukin 1 (IL-1), IL-6, IL-12, interferon alpha (IFN-α), interferon beta (IFN-β), and chemokines. Inflammatory disorders include, for example asthma, allergy, allergic rhinitis cardiovascular disease, chronic obstructive pulmonary disease (COPD), bronchiectasis, chronic cholecystitis, tuberculosis, Hashimoto's thyroiditis, sarcoidosis, silicosis and other pneumoconioses, and an implanted foreign body in a wound, but are not so limited.

In a further aspect of the invention a 2'-modified cytidine is provided for use in treatment of aberrant immune stimulation. In some instances the modification is a 2'-O-alkyl modification. In some instances the modification is a 2'-O-methyl modification. Other modificatications include but are not limited to 2'-O-ethyl, 2'-O-propyl, 2'-O-butyl or 2'-O-(2-methoxyethyl) modifications. Other useful modifications include 2'-O, 4'-C-alkylen-bridged nucleosides, such as. 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine. In some instances the 2'-O-alkyl-modification may contain at least one unsaturated carbon-carbon linkage such as a 2'-O-allyl or 2'-O-propinyl linkage. Additional examples include 2'-O-trifluoromethyl nucleosides, 2'-O-ethyl-trifluoromethoxy nucleosides, 2'-O-difluoromethoxy-ethoxy nucleosides. Examples of 2'-modified nucleosides and their synthesis are given for example in U.S. Application Serial No. 10/981,966, filed Nov. 5, 2004, and U.S. Patent No. 5859234, filed October 8, 1996, both of which are incorporated by reference herein). An example of the structure of one such 2'modified cytidine is as follows:



2'-O-Methyl Cytidine

The invention in another aspect provides a method for reducing an immunostimulatory effect of a TLR ligand or agonist. In some embodiments the TLR ligand can be a small molecule, a stimulatory ORN or a CpG nucleic acid molecule. The method involves the step of contacting an immune cell that is sensitive to the TLR ligand with an effective amount of an isolated immune modulatory ORN or 2' modified cytidine to reduce an immunostimulatory effect of the TLR ligand on the immune cell to a level below that which would occur without the contacting.

A further aspect of the invention is a modified ORN in which the ORN is modified on an rC residue. Such modifications according to the invention do not result in modified ORN with reduced immunostimulatory capacity or immune suppressive activity. In one aspect of the invention the ORN has unchanged or increased immune stimulatory capacity and can be used to stimulate an immune response in a subject in need of such treatment.

Autoimmune diseases can be generally classified as antibody-mediated, T-cell mediated, or a combination of antibody-mediated and T-cell mediated. Immune modulatory ORN of the invention are believed to be most useful for treating various types of autoimmunity involving antibody-mediated or T-cell mediated immunity, including insulin-dependent (type I) diabetes mellitus, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus (SLE), and inflammatory bowel disease (i.e., Crohn's disease and ulcerative colitis). Animal models for these autoimmune diseases are available and are useful for assessing the efficacy of inhibitory ODN in these diseases. Other autoimmune diseases include, without limitation, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, discoid lupus,

essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, juvenile arthritis, lichen planus, myasthenia gravis, polyarteritis nodosa, polychondritis, polyglandular syndromes, dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, sarcoidosis, stiff-man syndrome, Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

In several autoimmune diseases antibodies to self antigens are frequently observed. For example for systemic lupus erythematosus autoantibodies have been described to single-stranded and double-stranded DNA or RNA. Vallin H et al. (1999) *J Immunol* 163:6306-13; Hoet RM et al. (1999) *J Immunol* 163:3304-12; ven Venrooij (1990) *J Clin Invest* 86:2154-60. The levels of autoantibodies found in the serum of autoimmune patients very often are found to correlate with disease severity. The pattern of autoantibodies that arise, e.g., in human SLE, suggest that intact macromolecular particles, such as RNA- or DNA-containing complexes, could themselves be immunogenic and anti-nucleic acid antibodies could therefore arise. Lotz M et al. (1992) *Mol Biol Rep* 16:127; Mohan C et al. (1993) *J Exp Med* 177:1367-81. Such DNA or RNA released from, e.g., apoptotic cells or DNA- or RNA-containing microbes present in serum of autoimmune patients, could be responsible for inflammation that contributes to the autoimmune disease. Fatenejad S (1994) *J Immunol* 152:5523-31; Malmegrim KC et al. (2002) *Isr Med Assoc J* 4:706-12; Newkirk MM et al. (2001) *Arthritis Res* 3:253-8. Indeed CpG-containing sequences could be identified from SLE serum that induces an efficient immune response dominated by IFN-α secretion that is thought to contribute the development of to autoimmune diseases. Magnusson M et al. (2001) *Scand J Immunol* 54:543-50; Rönnblom L et al. (2001) *J Exp Med* 194:F59-63. In addition, the epitopes for anti-RNA antibodies could be identified and are composed of G,U-rich sequences. Tsai DE et al. (1992) *Proc Natl Acad Sci USA* 89:8864-8; Tsai DE et al. (1993) *J Immunol* 150:1137-45. G,U-rich sequences appear to be natural ligands for TLR7 and TLR8 and, therefore, can mediate immune stimulatory responses that in principle could contribute to autoimmune diseases or the development of autoimmune diseases. PCT/US03/10406. Indeed, RNA immune complexes and GU-rich sequences from such immune complexes that are targets for autoantibodies in SLE stimulate TLR7- and TLR8-mediated inflammatory responses. Vollmer et al. (2005), *J Exp Med* 202: 1575-1585.

Given the importance of immune stimulation mediated by serum CpG DNA or G,U-rich RNA that are targets for autoantibodies, the present invention provides a method for treating a condition associated with CpG DNA- or RNA-mediated immunostimulation in a subject having or being at risk of having an autoimmune disease.

In one embodiment the methods of the invention result in a shift in the immune system from a Th1-like immune response to a Th2-like immune response. A Th1-like immune response can include expression of any of certain cytokines and chemokines, including IFN-α, IFN-β, IFN-γ, TNF-α, IL-12, IL-18, IP-10, and any combination thereof, that are characteristically associated with a Th1 immune response. In some embodiments the Th2-like immune response can include induction of certain Th2-associated cytokines, including IL-4, IL-5, and IL-13. A Th2-like immune response can be useful in the treatment of any of a number of conditions that involve an innate immune response or a Th1-like immune response, including inflammation, acute and chronic allograft rejection, graft-versus-host disease (GvHD), certain autoimmune diseases, infection, and sepsis.

Infections refer to any condition in which there is an abnormal collection or population of viable intracellular or extracellular microbes in a subject. Various types of microbes can cause infection, including microbes that are bacteria, microbes that are viruses, microbes that are fungi, and microbes that are parasites.

Bacteria include, but are not limited to, *Pasteurella* species, *Staphylococci* species, *Streptococcus* species, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria include but are not limited to, *Helicobacter pyloris*, *Borrelia burgdorferi*, *Legionella pneumophilia*, *Mycobacteria* sps (e.g., *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. gordonae*), *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A Streptococcus), *Streptococcus agalactiae* (Group B Streptococcus), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic sps.), *Streptococcus pneumoniae*, pathogenic *Campylobacter sp.*, *Enterococcus sp.*, *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Corynebacterium sp.*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringens*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasturella multocida*, *Bacteroides sp.*, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidum*, *Treponema pertenue*, *Leptospira*, *Rickettsia*, and *Actinomyces israelii*.

Examples of viruses that have been found in humans include but are not limited to: *Retroviridae* (e.g., human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; *Picornaviridae* (e.g., polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); *Calciviridae* (e.g., strains that cause gastroenteritis); *Togaviridae* (e.g., equine encephalitis viruses, rubella viruses); *Flaviviridae* (e.g., dengue viruses, encephalitis viruses, yellow fever viruses); *Coronaviridae* (e.g., coronaviruses); *Rhabdoviridae* (e.g., vesicular stomatitis viruses, rabies viruses); *Filoviridae* (e.g., ebola viruses); *Paramyxoviridae* (e.g., parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); *Orthomyxoviridae* (e.g., influenza viruses); *Bunyaviridae* (e.g., Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); *Arenaviridae* (hemorrhagic fever viruses); *Reoviridae* (e.g., reoviruses, orbiviurses and rotaviruses); *Bornaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvoviridae* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses); *Adenoviridae* (most adenoviruses); *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); and *Iridoviridae* (e.g., African swine fever virus); and unclassified viruses (e.g., the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), Hepatitis C; Norwalk and related viruses, and astroviruses).

Fungi include yeasts and molds. Examples of fungi include without limitation *Aspergillus* spp including *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Candida* spp including *Candida albicans*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis carinii*, *Rhizomucor* spp, and *Rhizopus* spp.

Other infectious organisms (i.e., protists) include *Plasmodium spp.* such as *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium* vivax and *Toxoplasma gondii*. Blood-borne and/or tissue parasites include *Plasmodium* spp., *Babesia microti*, *Babesia divergens*, *Chlamydia trachomatis*, *Leishmania tropica*, *Leishmania* spp., *Leishmania braziliensis*, *Leishmania donovani*, *Trypanosoma gambiense* and *Trypanosoma rhodesiense* (African sleeping sickness), *Trypanosoma cruzi* (Chagas' disease), and *Toxoplasma gondii*.

Other medically relevant microorganisms have been described extensively in the literature, e.g., see C.G.A Thomas, *Medical Microbiology*, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference.

Sepsis is caused when severe infection over-activates the body's immune system, setting off a cascade of systemic inflammatory responses. Sepsis may be associated with clinical symptoms of systemic illness, such as fever, chills, malaise, low blood pressure, and mental status changes. Sepsis is potentially a life-threatening disease that can lead to a severe drop in blood pressure and cardiovascular collapse. Sepsis is most likely to develop in people with a weakened or underdeveloped immune system. Particularly at risk are those who are very young (particularly premature babies), are very old, are undergoing chemotherapy treatments, have AIDS, are undergoing organ transplant procedure, have wounds or injuries vulnerable to infection, have addictive habits such as alcohol or drug abuse, or are receiving treatments via intravenous catheters, wound drainage, urinary catheters, or other treatments which potentially allow bacteria access to the body.

The modified ORN of the instant invention can encompass various chemical modifications and substitutions, in addition to the 2' modification, in comparison to natural RNA and DNA, involving a phosphodiester internucleoside bridge, a β-D-ribose unit and/or a natural nucleoside base (adenine, guanine, cytosine, thymine, uracil). Examples of chemical modifications are known to the skilled person and are described, for example, in Uhlmann E et al. (1990) *Chem Rev* 90:543; "Protocols for Oligonucleotides and Analogs" Synthesis and Properties & Synthesis and Analytical Techniques, S. Agrawal, Ed, Humana Press, Totowa, USA 1993; Crooke ST et al. (1996) *Annu Rev Pharmacol Toxicol* 36:107-29; and Hunziker J et al. (1995) *Mod Synth Methods* 7:331-417. An oligonucleotide according to the invention may have one or more modifications, in addition to the 2' modification, wherein each modification is located at a particular phosphodiester internucleoside bridge and/or at a particular β-D-ribose unit and/or at a particular natural nucleoside base position in comparison to an oligonucleotide of the same sequence which is composed of natural DNA or RNA.

For example, the oligonucleotides may include one or more modifications and wherein each modification is independently selected from:

a) the replacement of a phosphodiester internucleoside bridge located at the 3′ and/or the 5′ end of a nucleoside by a modified internucleoside bridge,

b) the replacement of phosphodiester bridge located at the 3′ and/or the 5′ end of a nucleoside by a dephospho bridge,

c) the replacement of a sugar phosphate unit from the sugar phosphate backbone by another unit,

d) the replacement of a β-D-ribose unit by a modified sugar unit, and

e) the replacement of a natural nucleoside base by a modified nucleoside base.

More detailed examples for the chemical modification of an oligonucleotide are as follows.

The oligonucleotides may include modified internucleotide linkages, such as those described in a or b above. These modified linkages may be partially resistant to degradation (e.g., are stabilized). A stabilized oligonucleotide molecule is an oligonucleotide that is relatively resistant to in vivo degradation (e.g., via an exo- or endo-nuclease) resulting from such modifications. Oligonucleotides having phosphorothioate linkages, in some embodiments, may provide maximal activity and protect the oligonucleotide from degradation by intracellular exo- and endo-nucleases. As shown in the Examples, phosphorothioate molecules demonstrated better inhibitory activity than a corresponding phosphodiester molecule.

A phosphodiester internucleoside bridge located at the 3' and/or the 5' end of a nucleoside can be replaced by a modified internucleoside bridge, wherein the modified internucleoside bridge is for example selected from phosphorothioate, phosphorodithioate, NR^1R^2-phosphoramidate, boranophosphate, α-hydroxybenzyl phosphonate, phosphate-(C_1-C_{21})-O-alkyl ester, phosphate-[(C_6-C_{12})aryl-(C_1-C_{21})-O-alkyl]ester, (C_1-C_8)alkylphosphonate and/or (C_6-C_{12})arylphosphonate bridges, (C_7-C_{12})-α-hydroxymethyl-aryl (e.g., disclosed in WO 95/01363), wherein (C_6-C_{12})aryl, (C_6-C_{20})aryl and (C_6-C_{14})aryl are optionally substituted by halogen, alkyl, alkoxy, nitro, cyano, and where R^1 and R^2 are, independently of each other, hydrogen, (C_1-C_{18})-alkyl, (C_6-C_{20})-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, preferably hydrogen, (C_1-C_8)-alkyl, preferably (C_1-C_4)-alkyl and/or methoxyethyl, or R^1 and R^2 form, together with the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom from the group O, S and N.

The replacement of a phosphodiester bridge located at the 3' and/or the 5' end of a nucleoside by a dephospho bridge (dephospho bridges are described, for example, in Uhlmann E and Peyman A in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonucleotides and Analogs", S. Agrawal, Ed., Humana Press, Totowa 1993, Chapter 16, pp. 355 ff), wherein a dephospho bridge is for example selected from the dephospho bridges

formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethyl-hydrazo, dimethylenesulfone and/or silyl groups.

A sugar phosphate unit (i.e., a β-D-ribose and phosphodiester internucleoside bridge together forming a sugar phosphate unit) from the sugar phosphate backbone (i.e., a sugar phosphate backbone is composed of sugar phosphate units) can be replaced by another unit, wherein the other unit is for example suitable to build up a "morpholino-derivative" oligomer (as described, for example, in Stirchak EP et al. (1989) *Nucleic Acids Res* 17:6129-41), that is, e.g., the replacement by a morpholino-derivative unit; or to build up a polyamide nucleic acid ("PNA"; as described for example, in Nielsen PE et al. (1994) *Bioconjug Chem* 5:3-7), that is, e.g., the replacement by a PNA backbone unit, e.g., by 2-aminoethylglycine.

Nucleic acids also include substituted purines and pyrimidines such as C-5 propyne pyrimidine and 7-deaza-7-substituted purine modified bases. Wagner RW et al. (1996) *Nat Biotechnol* 14:840-4. Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, and thymine, and other naturally and non-naturally occurring nucleobases, substituted and unsubstituted aromatic moieties.

A modified base is any base which is chemically distinct from the naturally occurring bases typically found in DNA and RNA such as T, C, G, A, and U, but which share basic chemical structures with these naturally occurring bases. The modified nucleoside base may be, for example, selected from hypoxanthine, uracil, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(C_1-C_6)-alkyluracil, 5-(C_2-C_6)-alkenyluracil, 5-(C_2-C_6)-alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(C_1-C_6)-alkylcytosine, 5-(C_2-C_6)-alkenylcytosine, 5-(C_2-C_6)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine, 5-bromocytosine, N^2-dimethylguanine, 2,4-diamino-purine, 8-azapurine, a substituted 7-deazapurine, preferably 7-deaza-7-substituted and/or 7-deaza-8-substituted purine, 5-hydroxymethylcytosine, N4-alkylcytosine, e.g., N4-ethylcytosine, 5-hydroxydeoxycytidine, 5-hydroxymethyldeoxycytidine, N4-alkyldeoxycytidine, e.g., N4-ethyldeoxycytidine, 6-thiodeoxyguanosine, and deoxyribonucleosides of nitropyrrole, C5-propynylpyrimidine, and diaminopurine e.g., 2,6-diaminopurine, inosine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, hypoxanthine or other modifications of a natural nucleoside bases. This list is meant to be exemplary and is not to be interpreted to be limiting.

In particular formulas described herein modified bases may be incorporated. For instance a cytosine may be replaced with a modified cytosine. A modified cytosine as used herein is a naturally occurring or non-naturally occurring pyrimidine base analog of cytosine which can replace this base without impairing the immunostimulatory activity of the oligonucleotide. Modified cytosines include but are not limited to 5-substituted cytosines (e.g., 5-methyl-cytosine, 5-fluoro-cytosine, 5-chloro-cytosine, 5-bromo-cytosine, 5-iodo-cytosine, 5-hydroxy-cytosine, 5-hydroxymethyl-cytosine, 5-difluoromethyl-cytosine, and unsubstituted or substituted 5-alkynyl-cytosine), 6-substituted cytosines, N4-substituted cytosines (e.g., N4-ethyl-cytosine), 5-aza-cytosine, 2-mercapto-cytosine, isocytosine, pseudo-isocytosine, cytosine analogs with condensed ring systems (e.g., N,N'-propylene cytosine or phenoxazine), and uracil and its derivatives (e.g., 5-fluoro-uracil, 5-bromo-uracil, 5-bromovinyl-uracil, 4-thio-uracil, 5-hydroxy-uracil, 5-propynyl-uracil). Some of the preferred cytosines include 5-methyl-cytosine, 5-fluoro-cytosine, 5-hydroxy-cytosine, 5-hydroxymethyl-cytosine, and N4-ethyl-cytosine. In another embodiment of the invention, the cytosine base is substituted by a universal base (e.g., 3-nitropyrrole, P-base), an aromatic ring system (e.g., fluorobenzene or difluorobenzene) or a hydrogen atom (dSpacer).

A guanine may be replaced with a modified guanine base. A modified guanine as used herein is a naturally occurring or non-naturally occurring purine base analog of guanine which can replace this base without impairing the immunostimulatory activity of the oligonucleotide. Modified guanines include but are not limited to 7-deazaguanine, 7-deaza-7-substituted guanine (such as 7-deaza-7-(C2-C6)alkynylguanine), 7-deaza-8-substituted guanine, hypoxanthine, N2-substituted guanines (e.g., N2-methyl-guanine), 5-amino-3-methyl-3H,6H-thiazolo[4,5-d]pyrimidine-2,7-dione, 2,6-diaminopurine, 2-aminopurine, purine, indole, adenine, substituted adenines (e.g., N6-methyl-adenine, 8-oxo-adenine), 8-substituted guanine (e.g., 8-hydroxyguanine and 8-bromoguanine), and 6-thioguanine. In another embodiment of the invention, the guanine base is substituted by a universal base (e.g., 4-methyl-indole, 5-nitro-indole, and K-base), an aromatic ring system (e.g., benzimidazole or dichloro- benzimidazole, 1-methyl-1H-[1,2,4]triazole-3-carboxylic acid amide) or a hydrogen atom (dSpacer).

For use in the instant invention, the oligonucleotides of the invention can be synthesized de novo using any of a number of procedures well known in the art, for example, the β-cyanoethyl phosphoramidite method (Beaucage SL et al. (1981) *Tetrahedron Lett*

22:1859); or the nucleoside H-phosphonate method (Garegg et al. (1986) *Tetrahedron Lett* 27:4051-4; Froehler BC et al. (1986) *Nucleic Acids Res* 14:5399-407; Garegg et al. (1986) *Tetrahedron Lett* 27:4055-8; Gaffney et al. (1988) *Tetrahedron Lett* 29:2619-22). These chemistries can be performed by a variety of automated nucleic acid synthesizers available in the market. These oligonucleotides are referred to as synthetic oligonucleotides. An isolated oligonucleotide generally refers to an oligonucleotide which is separated from components which it is normally associated with in nature. As an example, an isolated oligonucleotide may be one which is separated from a cell, from a nucleus, from mitochondria or from chromatin.

Modified backbones such as phosphorothioates may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl-and alkyl-phosphonates can be made, e.g., as described in U.S. Pat. No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as described in U.S. Pat. No. 5,023,243 and European Patent No. 092,574) can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described (e.g., Uhlmann E et al. (1990) *Chem Rev* 90:544; Goodchild J (1990) *Bioconjugate Chem* 1:165).

In each of the foregoing aspects of the invention, the composition can also further include a pharmaceutically acceptable carrier, such that the invention also provides pharmaceutical compositions containing the isolated modified ORN or 2' modified nucleoside of the invention. As used herein, the term "pharmaceutically acceptable carrier" refers to one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. In some embodiments the carrier is a lipid carrier such as N-[1-(2,3-Dioleoyloxy)propyl]-N,N,Ntrimethylammoniummethyl-sulfate (DOTAP). DOTAP is believed to transport RNA oligomer into cells and specifically traffic to the endosomal compartment, where it can release the RNA oligomer in a pH-dependent fashion. Once in the endosomal compartment, the RNA can interact with certain intracellular TLRs, triggering TLR-mediated signal transduction pathways involved in modulating an immune response. Other agents with similar properties including trafficking to the endosomal compartment can be used in place of or in addition to DOTAP. Other lipid formulations include, for example, as EFFECTENE™ (a non-liposomal lipid with a special DNA condensing enhancer), SUPERFECT™ (a novel

acting dendrimeric technology), and Stable Nucleic Acid Lipid Particles (SNALPs) which employ a lipid bilayer. Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN™ and LIPOFECTACE™, which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes also have been reviewed by Gregoriadis G (1985) *Trends Biotechnol* 3:235-241. In some embodiments the pharmaceutically acceptable carrier is a cationic polymer, e.g. polyethylene imine (PEI), cyclodextrine, or chitosan.

The immune modulatory ORN of the invention can also be used for the preparation of a medicament for use in treatment of a condition in a subject. The use according to this aspect of the invention involves the step of placing an effective amount of a composition of the invention in a pharmaceutically acceptable carrier.

The term "effective amount" refers generally to the amount necessary or sufficient to realize a desired biologic effect. Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular oligonucleotide being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular immunosuppressive ORN and/or antigen and/or other therapeutic agent without necessitating undue experimentation.

For any compound described herein the therapeutically effective amount can be initially determined from animal models. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

For clinical use the modified ORN or 2' modified nucleoside of the invention can be administered alone or formulated as a delivery complex via any suitable route of

administration that is effective to achieve the desired therapeutic result. Routes of administration include enteral and parenteral routes of administration. Examples of enteral routes of administration include oral, gastric, intestinal, and rectal. Nonlimiting examples of parenteral routes of administration include intravenous, intramuscular, subcutaneous, intraperitoneal, intrathecal, local injection, topical, nasal, mucosal, and pulmonary.

The modified ORN or 2' modified nucleoside of the invention may be directly administered to the subject or may be administered in conjunction with a nucleic acid delivery complex. A nucleic acid delivery complex shall mean a nucleic acid molecule associated with (e.g., ionically or covalently bound to; or encapsulated within) a targeting means (e.g., a molecule that results in higher affinity binding to target cell. Examples of nucleic acid delivery complexes include nucleic acids associated with a sterol (e.g., cholesterol), a lipid (e.g., a cationic lipid, virosome or liposome such as DOTAP), or a target cell specific binding agent (e.g., a ligand recognized by target cell specific receptor). Preferred complexes may be sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex can be cleavable under appropriate conditions within the cell so that the oligonucleotide is released in a functional form.

For oral administration, the compounds (i.e., modified ORN or 2' modified nucleoside, antigens and/or other therapeutic agents) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may

also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds may be administered by inhalation to pulmonary tract, especially the bronchi and more particularly into the alveoli of the deep lung, using standard inhalation devices. The compounds may be delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. An inhalation apparatus may be used to deliver the compounds to a subject. An inhalation apparatus, as used herein, is any device for administering an aerosol, such as dry powdered form of the compounds. This type of equipment is well known in the art and has been described in detail, such as that description found in Remington: The Science and Practice of Pharmacy, 19th Edition, 1995, Mac Publishing Company, Easton, Pennsylvania, pages 1676-1692. Many U.S. patents also describe inhalation devices, such as U.S. Pat. No. 6,116,237.

"Powder" as used herein refers to a composition that consists of finely dispersed solid particles. Preferably the compounds are relatively free flowing and capable of being dispersed in an inhalation device and subsequently inhaled by a subject so that the compounds reach the lungs to permit penetration into the alveoli. A "dry powder" refers to a powder composition that has a moisture content such that the particles are readily dispersible in an inhalation device to form an aerosol. The moisture content is generally below about 10% by weight (% w) water, and in some embodiments is below about 5% w and preferably less than about 3% w. The powder may be formulated with polymers or optionally may be formulated with other materials such as liposomes, albumin and/or other carriers.

Aerosol dosage and delivery systems may be selected for a particular therapeutic application by one of skill in the art, such as described, for example in Gonda, I. "Aerosols for delivery of therapeutic and diagnostic agents to the respiratory tract," in Critical Reviews in Therapeutic Drug Carrier Systems, 6:273-313 (1990), and in Moren, "Aerosol dosage forms and formulations," in Aerosols in Medicine. Principles, Diagnosis and Therapy, Moren, et al., Eds., Elsevier, Amsterdam, 1985.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may include suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer R (1990) *Science* 249:1527-33, which is incorporated herein by reference.

The modified ORN or 2' modified nucleoside and optionally other therapeutics and/or antigens may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline

metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

The pharmaceutical compositions of the invention contain an effective amount of an modified ORN or 2' modified nucleoside and optionally antigens and/or other therapeutic agents optionally included in a pharmaceutically acceptable carrier. The term pharmaceutically acceptable carrier means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term carrier denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

The present invention is further illustrated by the following Examples, which in no way should be construed as further limiting.

EXAMPLES

Materials and Methods

Oligonucleotides and reagents

All ODN and ORN were purchased from Biospring (Frankfurt, Germany) or provided by Coley Pharmaceutical GmbH (Langenfeld, Germany), controlled for identity and purity by Coley Pharmaceutical GmbH and had undetectable endotoxin levels (<0.1EU/ml) measured by the Limulus assay (BioWhittaker, Verviers, Belgium). ODN were suspended in sterile, endotoxin-free Tris-EDTA (Sigma, Deisenhofen, Germany), ORN were suspended in sterile, DNAse- and RNAse-free dH_2O (Life Technologies, Eggenstein, Germany) and stored and handled under aseptic conditions to prevent both microbial and endotoxin contamination. All dilutions were carried out using endotoxin-free Tris-EDTA or DNAse- and RNAse-free

dH_2O. Nucleosides and chloroquine were obtained from Sigma or ChemGenes (Wilmington, MA, USA), and were dissolved in DMSO, NaOH or H_2O.

Cell purification

Peripheral blood buffy coat preparations from healthy human donors were obtained from the Blood Bank of the University of Düsseldorf (Germany) and PBMC were purified by centrifugation over Ficoll-Hypaque (Sigma). Cells were cultured in a humidified incubator at 37°C in RPMI 1640 medium supplemented with 5% (v/v) heat inactivated human AB serum (BioWhittaker) or 10% (v/v) heat inactivated FCS, 2mM L-glutamine, 100U/ml penicillin and 100μg/ml streptomycin (all from Sigma).

Cytokine detection

PBMC were resuspended at a concentration of $5x10^6$ cells/ml and added to 96 well round-bottomed plates (250μl/well). PBMC were incubated with various ODN, ORN or nucleoside concentrations and culture supernatants (SN) were collected after the indicated time points. If not used immediately, SN were stored at –20°C until required. For inhibitory experiments, cells were stimulated with the indicated TLR ligand concentration and nucleoside or ORN added. In some experiments, the second modified ORN was added 1h after the start of the cell culture.

Amounts of cytokines in the SN were assessed using a commercially available ELISA Kit for IL-12p40 (from BD Biosciences, Heidelberg, Germany), IFN-γ and TNF-α (from Diaclone, Besançon, France) or an in-house ELISA for IFN-α developed using commercially available antibodies (PBL, New Brunswick, NJ, USA). For analysis of a broad set of cytokines and chemokines, multiplex analysis with a luminex system from Bio-Rad (Munich, Germany) and Multiplex kits from Biosource (Solingen, Germany) was performed.

Naïve sv129 mouse splenocytes or the mouse macrophage cell line RAW264 were also used for in vitro cytokine induction. Animals were anesthetized with isofluorane and euthanized by cervical dislocation. Spleens were removed under aseptic conditions and placed in PBS + 0.2% BSA (Sigma, St. Louis, MO, USA). Spleens were then homogenized and splenocytes were re-suspended in RPMI 1640 (Life Technologies) medium supplemented with 2% normal mouse serum (Cedarlane Laboratories, Ontario, Canada), 2 mM L-

Glutamine, penicillin-streptomycin solution (final concentration of 1000 U/ml and 1 mg/ml respectively), and $5x10^{-5}$ M β-mercaptoethanol (all from Sigma). Splenocytes were plated in 96-well round-bottomed plates (5x105 cells/well). Each splenocyte sample was plated in quadruplicate and the cells were incubated in a humidified 5% CO2 incubator at 37°C for 20h. Supernatants were harvested and a commercially available assay kit for IL-6, IL-12p40 or TNF-α (mouse OptEIA kit; PharMingen, Mississauga, ON, Canada) was used according to manufacturers instructions to assay cytokine levels.

The following examples demonstrate the inhibitory effects of specific 2'-O-methyl modified nucleotides in a stimulatory ORN, as well as suppressive effects of 2'-O-methyl modified ORN and 2'OMe-C and –A nucleosides when added to stimulatory RNA or other TLR ligands as antagonistic molecules.

Example 1

2'-O-methylation interferes with RNA activation induced by self-RNA sequences or oligoribonucleotides containing immune stimulatory motifs

This set of experiments demonstrates the suppressive effects of 2' modified oligoribonucleotides (ORN) on RNA activation upon modification of the RNA molecule itself.

Cells were treated with various ORN and the resultant cytokine production was measured. The data in Figures 1a and 1b show interferon alpha (IFN-α) and tumor necrosis factor (TNF-α) production after treatment of cells with various oligoribonucleotides (see table 1). Oligoribonucleotides derived from the RNA sequence of the eukaryotic U1 snRNP particle were either unmodified or contained naturally occurring modifications present at the indicated positions (2'-O-methyl modification of adenosine (A) or uracil (U)). 2'-O-methylation of the unmodified stimulatory ORN SEQ ID NO:1, as shown for SEQ ID NO:9 (A and U), SEQ ID NO:2 (U) and SEQ ID NO:10 (A) resulted in a reduction of IFN-α and TNF-α compared to the unmodified SEQ ID NO:1. In data not shown a similar effect was observed for the U1 snRNA-derived ORN SEQ ID NO:11 and the same sequence with a 2' modification (SEQ ID NO:12) at a position that is found modified in the eukaryotic U1 snRNA. Most host-derived single-stranded RNA molecules contain a high frequency of such

modified nucleotides, and it is tempting to speculate that specific posttranscriptional modifications of host-derived RNA interferes with TLR-mediated effects and acts as a potential natural mechanism to prevent immune stimulation by self RNA. The data of Figures 1a and 1b demonstrate that the 2'-O-methyl modifications resulted in lower cytokine production.

Table 1: modified oligonucleotide sequences

SEQ ID NO:	SEQUENCE
1	rG*rA*rU*rA*rC*rU*rU*rA*rC*rC*rU*rG
2	rG*rA*mU*rA*rC*rU*rU*rA*rC*rC*rU*rG
9	rG*mA*mU*rA*rC*rU*rU*rA*rC*rC*rU*rG
10	rG*mA*rU*rA*rC*rU*rU*rA*rC*rC*rU*rG
11	rG*rG*rC*rU*rU*rA*rU*rC*rC*rA*rU*rU*rG*rC*rA*rC*rU*rC*rC*rG*rG*rA
12	rG*rG*rC*rU*rU*rA*rU*rC*rC*rA*rU*rU*rG*rC*mA*rC*rU*rC*rC*rG*rG

*** phosphorothioate linkage**

To investigate the effect of 2'-O-methyl modifications in more detail, various modifications were made to a simplified GU motif-containing RNA sequence (see table 2). The data in Figures 2a and 2b show that 2'-O-methyl modification of single nucleotides of the simplified immunostimulatory motif (UUGU) resulted in complete suppression of activation of IFN-α production and greatly reduced activation of TNF-α production. Similar results were observed in ORN of the same sequence with a phosphodiester backbone (data not shown).

Table 2: modified oligonucleotide sequences

SEQ ID NO:	SEQUENCE
15	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
16	rC*rC*rG*rA*rG*rC*rC*rG*rA*rA*rG*rG*rC*rA*rC*rC
17	rC*rC*rG*rA*rG*rC*rC*rG*rA*mU*rU*rG*rU*rA*rC*rC
18	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*mU*rG*rU*rA*rC*rC
19	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*mG*rU*rA*rC*rC
20	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*mU*rA*rC*rC

*** phosphorothioate linkage**

Example 2

2'-O-methylation outside the immune stimulatory motif interferes with RNA activation

In order to determine whether modification of residues outside the immune stimulatory GU motif would result in a reduction in immune modulatory activity of the ORN, 2'O-methyl modifications were introduced at various positions of the ORN (see table 3). The

data in Figures 3a and 3b demonstrate that the negative effect of single nucleotide modifications in a stimulatory single stranded ORN can be attributed to modification of only rA, rU, and rG. Surprisingly, 2' modification of rC residues at different positions in the ORN (SEQ ID NO:13, SEQ ID NO:14) did not result in a decrease of the ORN-mediated effects..

Table 3: modifications outside ORN stimulatory motif

SEQ ID NO	SEQUENCE
13	mC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
14	rC*rC*rG*rA*mG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
15	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
21	rC*rC*rG*rA*rG*rC*rC*rG*mA*rU*rU*rG*rU*rA*rC*rC
22	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*mA*rC*rC
23	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*mC

* phosphorothioate linkage

The data in Figures 4a and 4b demonstrate that while modifications 3' and 5' of the immune stimulatory motif (see table 4) resulted in similar effects on immune modulation, the degree of the negative effect on immune modulation was influenced by the particular residue that had been modified. All ORN with rC modifications tested did show similar activity to the unmodified parent ORN (SEQ ID NO:15), although all other ORN with modifications at rG or rA tested did have decreased immune effects.

Table 4: modifications outside ORN stimulatory motif

SEQ ID NO	SEQUENCE
15	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
21	rC*rC*rG*rA*rG*rC*rC*rG*mA*rU*rU*rG*rU*rA*rC*rC
22	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*mA*rC*rC
24	rC*rC*mG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
25	rC*rC*rG*rA*rG*rC*mC*rG*rA*rU*rU*rG*rU*rA*rC*rC
26	rC*rC*rG*rA*rG*rC*rC*mG*rA*rU*rU*rG*rU*rA*rC*rC
27	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*mC*rC

* phosphorothioate linkage

To further confirm the unexpected lack of the described effect of rC versus rG or rA modification, additional ORN with 2' modification at the rC at other positions than the previously used ORN were tested for their immune stimulatory effects. Figures 5a and 5b demonstrate that as described before 2'-O-methyl A modifications had a strong effect on immune stimulation, whereas modified rC at positions directly adjacent to the immune stimulatory GU motif showed no significant difference in immune stimulation to the unmodified ORN (Figure 5 and table 5).

Table 5: modifications outside ORN stimulatory motif

SEQ ID NO	SEQUENCE
15	rC*rC*rG*rA*rG*rC*rC*rG*rA*rU*rU*rG*rU*rA*rC*rC
28	rC*rC*rG*rA*rG*rC*rC*rG*rC*rU*rU*rG*rU*rC*rC*rC
29	rC*rC*rG*rA*rG*rC*rC*rG*mC*rU*rU*rG*rU*rC*rC*rC
30	rC*rC*rG*rA*rG*rC*rC*rG*rC*rU*rU*rG*rU*mC*rC*rC

* phosphorothioate linkage

Example 3

2'-O-methylation of ORN results in suppressive ORN inhibiting RNA- and DNA-mediated immune stimulation

Phosphorothioate ORN with 2' modifications at rA, rG or rU, but not rC resulted even in suppression of stimulatory ORN effects upon co-culture. A study was performed in order to test the effect of 2'-modified ORN on immune cell activation by an immune stimulatory ORN (see table 6). The data in Figures 6a and 6c show the effects of the immune stimulatory ORN SEQ ID NO:1 on activation of IFN-α and TNF-α, respectively. 2'-O-methyl modification of a rU in ORN SEQ ID NO:1 (producing ORN SEQ ID NO:2) resulted in an ORN that showed significantly decreased induction of IFN-α and TNF-α. Surprisingly, the 2' modification in ORN SEQ ID NO:1 (resulting in ORN SEQ ID NO:2) did not only inhibit its own immune modulatory effects (Figures 6a and 6c), but did suppress the stimulatory effects of the stimulatory unmodified ORN upon co-culture (SEQ ID NO:1) (Figures 6b and 6d), probably acting as a TLR antagonist. Similar results were observed for all cytokines tested, IFN-α, TNF-α, IFN-γ and IL-12 (Figures 7 and 8).

More importantly, ORN SEQ ID NO:1 is derived from a naturally occurring RNA, the U1 snRNA. U1 snRNA-containing immune complexes (U1 snRNP) were demonstrated to be involved in autoimmune responses such as SLE, and autoantibodies targeting snRNPs and the snRNA can be observed in SLE patients. In addition, the inflammation observed in SLE patients may be attributed to stimulatory effects induced by the snRNA, as the U1 snRNA itself as well as GU-containing sequences derived from this snRNA such as ORN SEQ ID NO:1 were demonstrated to be immune modulatory. Therefore, the suppressive ORN may be used to reduce or suppress inflammatory responses mediated by self RNA such as the U1 snRNA.

Table 6: Immune stimulatory and modified ORN

SEQ ID NO	SEQUENCE
1	rG*rA*rU*rA*rC*rU*rU*rA*rC*rC*rU*rG
2	rG*rA*mU*rA*rC*rU*rU*rA*rC*rC*rU*rG

SEQ ID NO	SEQUENCE
1	rG*rA*rU*rA*rC*rU*rU*rA*rC*rC*rU*rG
2	rG*rA*mU*rA*rC*rU*rU*rA*rC*rC*rU*rG

*** phosphorothioate linkage**

Moreover, the antagonistic effect was not only observed with ORN SEQ ID NO:1 as a stimulatory ORN, but could be reproduced with a variety of other stimulatory ORN. One example is given in Figure 8. The immune stimulatory ORN SEQ ID NO:7 containing a GU immune stimulatory motif was co-cultured with the 2' rU modified ORN SEQ ID NO:2. SEQ ID NO:2 did suppress the immune stimulatory effects of ORN SEQ ID NO:4 similar to SEQ ID NO:1 (Figure 8 and Figure 6, respectively). In addition, it was tested whether the phosphorothioate 2'-modified ORN SEQ ID NO:2 was also able to suppress the effects of an ORN with the naturally occurring phosphodiester backbone (ORN SEQ ID NO:5 in Figure 8b). Although showing a slightly different dose-response curve, the suppressive ORN SEQ ID NO:2 was able to inhibit the activity of the same sequence with a phosphorothioate (SEQ ID NO:7) or phosphodiester (SEQ ID NO:5) backbone. In addition, strength of inhibitory effect depended on the backbone chemistry. The 2' modified sequence of ORN SEQ ID NO:2 with a phosphodiester backbone (in ORN SEQ ID NO:6) did suppress the activity of the stimulatory ORN SEQ ID NO:7 only at the highest concentrations used (Figure 8a), indicating that, although being a suppressor of cytokine production, the phosphodiester sequence may be not stable enough and may be degraded before substantially interfering with the immune stimulatory effects. The suppressive effect of the 2' modified ORN was also observed when the inhibitory ORN SEQ ID NO:2 was added to the cells 1h after the stimulatory ORN SEQ ID NO:1 (data not shown), and, therefore, appears not to be attributable to uptake competition.

Table 7: Immune stimulatory and modified ORN with PO and PS backbones

SEQ ID NO	SEQUENCE
7	rC*rC*rG*rU*rC*rU*rG*rU*rU*rG*rU*rG*rU*rG*rA*rC*rU*rC
5	rC-rC-rG-rU-rC-rU-rG-rU-rU-rG-rU-rG-rU-rG-rA-rC-rU-rC
2	rG*rA*mU*rA*rC*rU*rU*rA*rC*rC*rU*rG
6	rG-rA-mU-rA-rC-rU-rU-rA-rC-rC-rU-rG

*** phosphorothioate linkage**

-*phosphodiester linkage

To further test if the suppressive effect was indeed attributable to the 2' modification, we compared the effect of an unmodified ORN SEQ ID NO:16 with a modified ORN SEQ ID NO:8 (with the same sequence as SEQ ID NO:16, and SEQ ID NO:2 (Figure 9a and Figure 9b). Although the unmodified ORN SEQ ID NO:16 had some inhibitory effect on IFN-α production (Figure 9a) induced by stimulatory ORN SEQ ID NO:4, probably due to uptake competition, ORN SEQ ID NO:2 and especially SEQ ID NO:8 did substantially suppress the stimulatory effects of ORN SEQ ID NO:7 for IFN-α (Figure 9a) and TNF-α (Figure 9b) induction, demonstrating that the 2' modification is responsible for the suppressive effects.

The effects observed so far related to the suppression of TLR7,8-dependent immune stimulation. TLR7 and TLR8 are receptors responding to stimulation with RNA or ORN. Therefore, a potential suppressive effect of 2' modified ORN on other than TLR7,8-mediated effects was investigated. TLR9 is the receptor for DNA containing CpG dinucleotides. The unmodified ORN SEQ ID NO:16 did not exhibit an effect on the IFN-α response induced by C-Class CpG ODN SEQ ID NO:4 in the presence of DOTAP (Figure 10). Nevertheless, a clear shift of the SEQ ID NO:4-mediated response, and therefore interference with and inhibition of CpG-mediated effects, was observed, although not complete. It may be possible that higher concentrations of the suppressive ORN SEQ ID NO:2 would result in complete inhibition of the CpG SEQ ID NO:9-mediated immune response.

Example 4

Single 2'-O-methyl cytidine nucleosides exhibit inhibitory activity

In order to test whether a single 2'-O-methyl nucleoside could suppress the immune stimulatory capacity of an immunostimulatory ORN, 2'-O-methyl C, A, U, and G nucleosides were co-incubated with an immunostimulatory RNA and cytokine production was monitored. 2'-O-methyl C nucleosides resulted in significant inhibition of IFN-α production. Slight inhibition of IFN-α production was also seen with 2'-O-methyl A nucleosides (Figure 11a). Surprisingly, the 2'-O-Me-modified C nucleoside was not capable at these concentrations of suppressing the stimulation of other cytokines such as TNF-α and IL-12. A suppressive effect could be observed for IFN-γ, although not as strong. GU-containing ORN specifically

activate pDC that express TLR7, but not TLR8, to produce IFN-α, whereas other cells such as TLR8-expressing monocytes are stimulated to produce TNF-α, IFN-γ or IL-12. Therefore, it appears possible that the stronger inhibitory effect is exerted on pDC and IFN-α induction compared to IFN-γ or IL-12 producing cells. The data of Figure 11 demonstrate that 2'-O-methyl C nucleoside specifically inhibits ORN-mediated IFN-α and to some lesser degree IFN-γ production. Moreover, in data not shown the inhibitory capacity of a 2'-O-Me modified rC and an unmodified rC was compared. The results demonstrate that the 2' modification is responsible for the suppressive effect as the unmodified rC had some effect only at the highest concentration tested (100μg).

Two other suppressors of TLR-dependent signaling were previously described, S-Class ODN SEQ ID NO:3 and chloroquine. Both molecules appear to act in an antagonistic way at the receptor level. Suppression mediated by these molecules and a 2' modified ORN (SEQ ID NO:2) as well as a 2' modified C nucleoside (2'-O-Me-C) was compared (Figure 11a). The data indicate that chloroquine and 2'-O-Me-C exhibit similar inhibitory activity that was slightly better than the 2' modified ORN, and was stronger than the suppression mediated by S-Class ODN SEQ ID NO:3. Therefore, 2'-O-Me-C and 2' modified ORN decrease the RNA-mediated response in a manner similar to the well known TLR antagonists chloroquine and S-Class ODN SEQ ID NO:3.

To further investigate if the 2' modified C is suppressive to the stimulation via other TLRs, the TLR4 ligand LPS as well as the TLR9 ligand C-Class CpG ODN SEQ ID NO:4 were used. Figure 12 shows that 2'-O-Me-C was not able to suppress the TNF-α induction mediated by LPS, although 2'-O-Me-A showed some effect in line with the previously reported negative effect of adenosine and analogs on TLR cytokine production.

Similar tests were performed to analyze the effect on CpG-mediated cytokine production. As shown in Figure 13, stimulation of IFN-α production mediated by C-Class CpG ODN SEQ ID NO:4 was decreased by 2'-O-methyl C in a manner comparable to the known inhibitor of TLR9-mediated effects, chloroquine. Some inhibition could also be observed for 2'-O-Me-A indicating that 2'-O-Me-A exhibits its inhibitory effect not only on TLR4, LPS-mediated TNF-α induction as observed before, but also on cytokine induction induced by TLR7,8,9 ligands (GU ORN and CpG ODN).

Figure 14 shows a comparison of the inhibitory effects on TLR7/8 (ORN SEQ ID NO:1), TLR9 (C-Class CpG ODN SEQ ID NO:4)and TLR4 (LPS) ligand activity for a panel

of cytokines and chemokines. The figure shows the % inhibition mediated by the inhibitory molecule (2'-O-Me-C, chloroquine or S-Class ODN SEQ ID NO:3) of the activity of the TLR ligand alone set to 100%. Chloroquine at the concentration used suppressed the ORN-dependent effects for all cytokines nearly to 100% (Figure 14a). The 2'-O-Me-C was not as suppressive for most of the ORN-mediated effects, as expected from the previous experiments demonstrating an inhibitory effect mainly on IFN-α and some effect on IFN-γ. Indeed, from all the cytokines and chemokines shown, the strongest suppressive effects could only be observed for IFN-α and IFN-γ, and some effect also for e.g., RANTES and IP-10. Therefore, these data confirm the previous experiments demonstrating that 2'-O-Me-C is an inhibitor of the ORN-mediated IFN-α response. In addition, the suppressive effect of chloroquine, 2'-O-Me-C and S-Class ODN SEQ ID NO:3 on the response induced by the TLR9 ligand C-Class ODN SEQ ID NO:4 was tested (Figure 14b). As observed for the RNA-mediated effects, chloroquine was a strong suppressor of the CpG-mediated cytokines and chemokines. In contrast, 2'-O-Me-C was as good a suppressor for all cytokines and chemokines induced by the CpG ODN tested, and even stronger for some effects such as IL-2R and MCP-1 induction. In contrast to these observations, 2'-O-Me-C was not capable of suppressing substantially the effects induced by LPS (Figure 14c). In summary, 2'O-methyl C demonstrated strongest inhibitory activity toward CpG SEQ ID NO:4. It is also suppressive to ORN SEQ ID NO:1.

Example 5

Effect of 2'-O-methylation on murine TLR7-dependent immune responses

In order to test the suppressive effect of the 2'-O-Me modification on murine TLR7, murine splenocytes or RAW264 murine macrophages were stimulated for 20h with 1.0μM (splenocytes) or 0.25μM (RAW264) ORN SEQ ID NO:7 complexed to DOTAP, or in the presence of the indicated concentrations of the unmodified non-stimulatory ORN SEQ ID NO:16, or the same sequence with a single 2'-O-methyl modification (SEQ ID NO:8), and cytokines measured. Figure 15 shows induction of TNF-α in RAW cells. As shown in Figure 17, 2'-O-Me modified SEQ ID NO:8 and non-stimulatory SEQ ID NO:16 did not induce TNF-α (second and third panel). These ORN had no suppressive effects when combined with stimulatory ORN SEQ ID NO:7 (sixth and seventh panel).

In order to test the suppressive effect of the 2'-O-Me modification on IL-12 (Figure 16a) and IL-6 (Figure 16b) induction in murine spelenocytes. Cells were treated with stimulatory ORN SEQ ID NO:7 along with either the unmodified non-stimulatory ORN SEQ ID NO:16, or the same sequence with a single 2'-O-methyl modification (SEQ ID NO:8) at the concentrations indicated, and concentration of cytokines in the supernatants were measured. Cells treated with SEQ ID NO:8 produced less IL-12 and IL-6 than cells treated with the non-stimulatory ORN SEQ ID NO:16, demonstrating that the effect is a true suppressive effect rather than competition with the stimulatory ORN SEQ ID NO:7.

Table 8: Immune stimulatory and modified ORN

SEQ ID NO	SEQUENCE
7	rC*rC*rG*rU*rC*rU*rG*rU*rU*rG*rU*rG*rU*rG*rA*rC*rU*rC
8	rC*rC*rG*rA*rG*rC*rC*rG***mA***rA*rG*rG*rC*rA*rC*rC
16	rC*rC*rG*rA*rG*rC*rC*rG*rA*rA*rG*rG*rC*rA*rC*rC

*** phosphorothioate linkage**

EQUIVALENTS

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

We claim:

CLAIMS

1. A method for treating autoimmune disease in a subject, comprising administering to a subject in need of such treatment an effective amount for treating autoimmune disease of a modified oligoribonucleotide having an immune modulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a nucleoside 3' or 5'of the immune modulatory motif.

2. The method of claim 1 wherein the 2' modification is within 9 nucleotides of the immune modulatory motif.

3. The method of claim 1 wherein the 2' modification decreases immune modulatory activity of the ORN containing the motif.

4. The method of claim 1 wherein the autoimmune disease involves antibody-mediated or T-cell mediated immunity.

5. The method of claim 1 wherein the autoimmune disease is selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome,

systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

6. The method of claim 1 wherein the 2' modification is on a rA, rG or rU residue.

7. The method of claim 1 wherein the 2' modification is O-methyl.

8. The method of claim 1 wherein the nucleobase of the modified residue is selected from a group consisting of hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(C1-C6)-alkylcytosine, 5-methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine, 5-bromocytosine, N2-dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4-diaminopurine, 2,6-diaminopurine, 8-azapurine, substituted 7-deazapurine, 7-deaza-7-substituted purine, 7-deaza-8-substituted purine, hydrogen (abasic residue).

9. The method of claim 1 wherein the immune modulatory motif has a base sequence selected from

(i) 5' U U G U 3'
(ii) 5' C/U U G/U U 3',
(iii) 5' R U R G Y 3',
(iv) 5' G U U G B 3',
(v) 5' G U G U G/U 3',
(vi) 5' G/C U A/C G G C A C 3', and
(vii) N-U-R1-R2,

wherein C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and wherein at least one of R1 and R2 is adenosine (A) or

cytosine (C) or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A.

10. The method of claim 1 wherein the subject is a subject having an autoimmune disease.

11. The method of claim 1 wherein the subject is a subject at risk of developing a autoimmune disease.

12. The method of claim 1 wherein the modified oligoribonucleotide is single stranded and wherein the oligoribonucleotide sequence is not complementary to a coding sequence in the target cell.

13. The method of claim 1 wherein the immune modulatory motif comprises at least one 2' modified nucleoside.

14. A method for treating an inflammatory disorder in a subject, comprising administering to a subject in need of such treatment an effective amount for treating an inflammatory disorder of a modified oligoribonucleotide having an immune modulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a nucleoside 3' or 5'of the immune modulatory motif.

15. The method of claim 14 wherein the subject is a subject at risk of developing an inflammatory disorder.

16. The method of claim 14 wherein the inflammatory disorder is sepsis.

17. The method of claim 14 wherein the inflammatory disorder is an infection.

18. The method of claim 14 wherein the 2' modification is on a rA, rG or rU residue.

19. The method of claim 14 wherein the 2' modification is O-methyl.

20. The method of claim 14 wherein the nucleobase of the modified residue is selected from a group consisting of hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2 thiouracil, 4 thiouracil, 5 aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5 (hydroxymethyl)uracil, 5 chlorouracil, 5 fluorouracil, 5 bromouracil, 5 hydroxycytosine, 5-(C1-C6)-alkylcytosine, 5 methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5 chlorocytosine, 5 fluorocytosine, 5 bromocytosine, N2 dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4 diaminopurine, 2,6-diaminopurine, 8 azapurine, substituted 7 deazapurine, 7 deaza 7 substituted purine, 7 deaza 8 substituted purine, hydrogen (abasic residue).

21. The method of claim 14 wherein the immune modulatory motif has a base sequence selected from

(i) 5' U U G U 3'
(ii) 5' C/U U G/U U 3',
(iii) 5' R U R G Y 3',
(iv) 5' G U U G B 3',
(v) 5' G U G U G/U 3',
(vi) 5' G/C U A/C G G C A C 3', and
(vii) N-U-R1-R2,

wherein C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and wherein at least one of R1 and R2 is adenosine (A) or cytosine (C) or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A.

22. The method of claim 14 wherein the immune modulatory motif comprises at least one 2' modified nucleoside.

23. A composition comprising a modified oligoribonucleotide, wherein the modified oligoribonucleotide contains at least one 2'modification on a residue 3' or 5'of an immune modulatory motif, wherein the 2' modification is on a rA, rG or rU residue.

24. The composition of claim 23 wherein the 2' modification is O-methyl.

25. The composition of claim 23 wherein the nucleobase of the modified residue is selected from a group consisting of hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2 thiouracil, 4 thiouracil, 5 aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5 (hydroxymethyl)uracil, 5 chlorouracil, 5 fluorouracil, 5 bromouracil, 5 hydroxycytosine, 5-(C1-C6)-alkylcytosine, 5 methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5 chlorocytosine, 5 fluorocytosine, 5 bromocytosine, N2 dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4 diaminopurine, 2,6-diaminopurine, 8 azapurine, substituted 7 deazapurine, 7 deaza 7 substituted purine, 7 deaza 8 substituted purine, hydrogen (abasic residue).

26. The composition of claim 23 wherein the modified oligoribonucleotide has a backbone modification.

27. The composition of claim 26 wherein the backbone modification is a phosphorothioate modification.

28. The composition of claim 23 wherein the modified oligoribonucleotide is between 10 and 30 nucleotides in length.

29. The composition of claim 23 wherein the modified oligoribonucleotide contains at least two modified residues.

30. The composition of claim 23 wherein the modified oligoribonucleotide contains at least three modified residues.

31. The composition of claim 23 wherein the immune modulatory motif has a base sequence selected from

(i) 5' U U G U 3'
(ii) 5' C/U U G/U U 3',
(iii) 5' R U R G Y 3',
(iv) 5' G U U G B 3',
(v) 5' G U G U G/U 3',
(vi) 5' G/C U A/C G G C A C 3', and
(vii) N-U-R1-R2,

wherein C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and wherein at least one of R1 and R2 is Adenosine (A) or Cytosine or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A.

32. The composition of claim 23 wherein the modified oligoribonucleotide is single stranded and wherein the oligoribonucleotide sequence is not complementary to a coding sequence in the target cell.

33. The composition of claim 23 wherein the modified oligoribonucleotide comprises at least one 2' modified nucleoside in the immune modulatory motif.

34. A method for suppressing an immune response in a subject, comprising administering to a subject in need of such treatment a modified oligoribonucleotide of any one of claims 23-31.

35. The method of claim 34 wherein the immune response is an RNA-mediated immune response.

36. The method of claim 34 wherein the immune response is a DNA-mediated immune response.

37. The method of claim 34 wherein the subject has an autoimmune disease.

38. The method of claim 34 wherein the subject is at risk of developing an autoimmune disease.

39. The method of claim 34 wherein the subject has an inflammatory disorder.

40. The method of claim 34 wherein the suppression of the immune response comprises suppression of TLR8 signaling.

41. The method of claim 34 wherein the suppression of the immune response comprises suppression of TLR7 signaling.

42. The method of claim 34 wherein the suppression of the immune response comprises suppression of TLR9 signaling.

43. The method of claim 34 wherein the suppression of the immune response comprises suppression of activation of antigen-presenting cells, B cells, myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), monocytes, monocyte-derived cells, eosinophils, or neutrophils.

44. The method of claim 34, wherein the subject is administered a TLR ligand.

45. The method of claim 44 wherein the TLR ligand is a CpG oligonucleotide.

46. The method of claim 44 wherein the TLR ligand is an immune stimulatory RNA.

47. The method of claim 43 wherein the modified oligoribonucleotide is single stranded and wherein the oligoribonucleotide sequence is not complementary to a coding sequence in the target cell.

48. The method of claim 44 wherein the ligand is a small molecule.

49. A method of inhibiting an RNA-mediated immune response in a subject, comprising administering to a subject in need of such treatment a 2'-modified cytidine.

50. The method of claim 49 wherein the 2'-modified cytidine is 2'-O-methyl cytidine.

51. The method of claim 49 wherein the 2'-modified cytidine is 2'-O-alkyl cytidine.

52. The method of claim 49 wherein the 2'-modified cytidine is selected from the group comprising 2'-O-ethyl, 2'-O-propyl and 2'-O-butyl cytidine.

53. The method of claim 49 wherein the 2'-modified cytidine is a 2'-O, 4'-C-alkylen-bridged nucleoside.

54. The method of claim 53 wherein the nucleoside is a 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine.

55. The method of claim 49 wherein the 2'-modified cytidine contains at least one unsaturated carbon-carbon linkage, e.g. 2'-O-allyl or 2'-O-propinyl.

56. The method of claim 51 wherein the 2'-O-alkyl-modification is 2'-O-(2-methoxyethyl).

57. The method of claim 49 wherein the subject is a subject having or at risk of having a condition selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune

hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

58. A method of inhibiting a DNA-mediated immune response in a subject, comprising administering to a subject in need of such treatment a 2'-modified cytidine.

59. The method of claim 58 wherein the 2'-modified cytidine is 2'-O-methyl cytidine.

60. The method of claim 58 wherein the 2'-modified cytidine is 2'-O-alkyl cytidine.

61. The method of claim 58 wherein the 2'-modified cytidine is selected from the group comprising 2'-O-ethyl, 2'-O-propyl and 2'-O-butyl cytidine.

62. The method of claim 58 wherein the 2'-modified cytidine is a 2'-O, 4'-C-alkylen-bridged nucleoside.

63. The method of claim 62 wherein the nucleoside is a 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine.

64. The method of claim 58 wherein the 2'-modified cytidine contains at least one unsaturated carbon-carbon linkage, e.g. 2'-O-allyl or 2'-O-propinyl.

65. The method of claim 60 wherein the 2'-O-alkyl-modification is 2'-O-(2-methoxyethyl).

66. The method of claim 58 wherein the subject is a subject having or at risk of having a condition selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

67. A method of treating an autoimmune response in a subject, comprising administering to a subject in need of such treatment a 2'-modified cytidine.

68. The method of claim 67 wherein the 2'-modified cytidine is 2'-O-methyl cytidine.

69. The method of claim 67 wherein the 2'-modified cytidine is 2'-O-alkyl cytidine.

70. The method of claim 67 wherein the 2'-modified cytidine is selected from the group comprising 2'-O-ethyl, 2'-O-propyl and 2'-O-butyl cytidine.

71. The method of claim 67 wherein the 2'-modified cytidine is a 2'-O, 4'-C-alkylen-bridged nucleoside.

72. The method of claim 71 wherein the nucleoside is a 2'-O, 4'-C-methylen-bridged cytidine (LNA analogue of cytidine) or 2'-O, 4'-C-ethylen-bridged cytidine.

73. The method of claim 67 wherein the 2'-modified cytidine contains at least one unsaturated carbon-carbon linkage, e.g. 2'-O-allyl or 2'-O-propinyl.

74. The method of claim 69 wherein the 2'-O-alkyl-modification is 2'-O-(2-methoxyethyl).

75. The method of claim 67 wherein the subject is a subject having or at risk of having a condition selected from the group comprising scleroderma, juvenile rheumatoid arthritis, ulcerative colitis, graft versus host disease, transplanted organ rejection, asthma, alopecia areata, acquired hemophilia, ankylosing spondylitis, antiphospholipid syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, Behçet's syndrome, cardiomyopathy, celiac sprue dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia, fibromyositis, Guillain-Barré syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), juvenile arthritis, lichen planus, myasthenia gravis, multiple sclerosis, mixed connective tissue disease, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomena, Reiter's syndrome, rheumatoid arthritis (RA), Sjorgen's syndrome, sarcoidosis, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arthritis, temporal arteritis/giant cell arteritis, uveitis, vasculitis, and vitiligo.

76. Use of a compound comprising a modified oligoribonucleotide having an immune modulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a

nucleoside 3' or 5'of the immune modulatory motif, for the manufacture of a pharmaceutical composition for treatment of autoimmune disease.

77. Use of a compound comprising a modified oligoribonucleotide having an immune modulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a nucleoside 3' or 5'of the immune modulatory motif, for the manufacture of a pharmaceutical composition for treatment of an inflammatory disorder.

78. Use of a compound comprising a 2'-modified cytidine, for the manufacture of a pharmaceutical composition for inhibiting an RNA-mediated immune response.

79. Use of a compound comprising a 2'-modified cytidine, for the manufacture of a pharmaceutical composition for inhibiting a DNA-mediated immune response in a subject.

80. Use of a compound comprising a 2'-modified cytidine, for the manufacture of a pharmaceutical composition for treatment of autoimmune disease.

81. Use of a compound comprising a modified oligoribonucleotide having an immune stimulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a rC residue 3' or 5'of the immune stimulatory motif, for the manufacture of a pharmaceutical composition for stimulating an immune response.

82. A method for stimulating an immune response, comprising, administering to a subject an effective amount for stimulating an immune response in the subject of a modified oligoribonucleotide having an immune stimulatory motif 4 to 8 nucleotides long and including at least one 2' modification on a rC residue 3' or 5'of the immune stimulatory motif.

83. The method of claim 82, wherein the immune stimulatory motif has a base sequence selected from

(i) 5' U U G U 3'

(ii) 5' C/U U G/U U 3',
(iii) 5' R U R G Y 3',
(iv) 5' G U U G B 3',
(v) 5' G U G U G/U 3',
(vi) 5' G/C U A/C G G C A C 3', and
(vii) N-U-R1-R2,

wherein C/U is cytosine (C) or uracil (U), G/U is guanine (G) or U, R is purine, Y is pyrimidine, B is U, G, or C, G/C is G or C, A/C is adenine (A) or C, N is a ribonucleoside and N does not include a U, and wherein at least one of R1 and R2 is Adenosine (A) or Cytosine or derivatives thereof and wherein R is not U unless N-U-R1-R2 includes at least two A.

84. The method of claim 82 wherein the modified oligoribonucleotide comprises at least one 2' modified nucleoside in the immune modulatory motif.

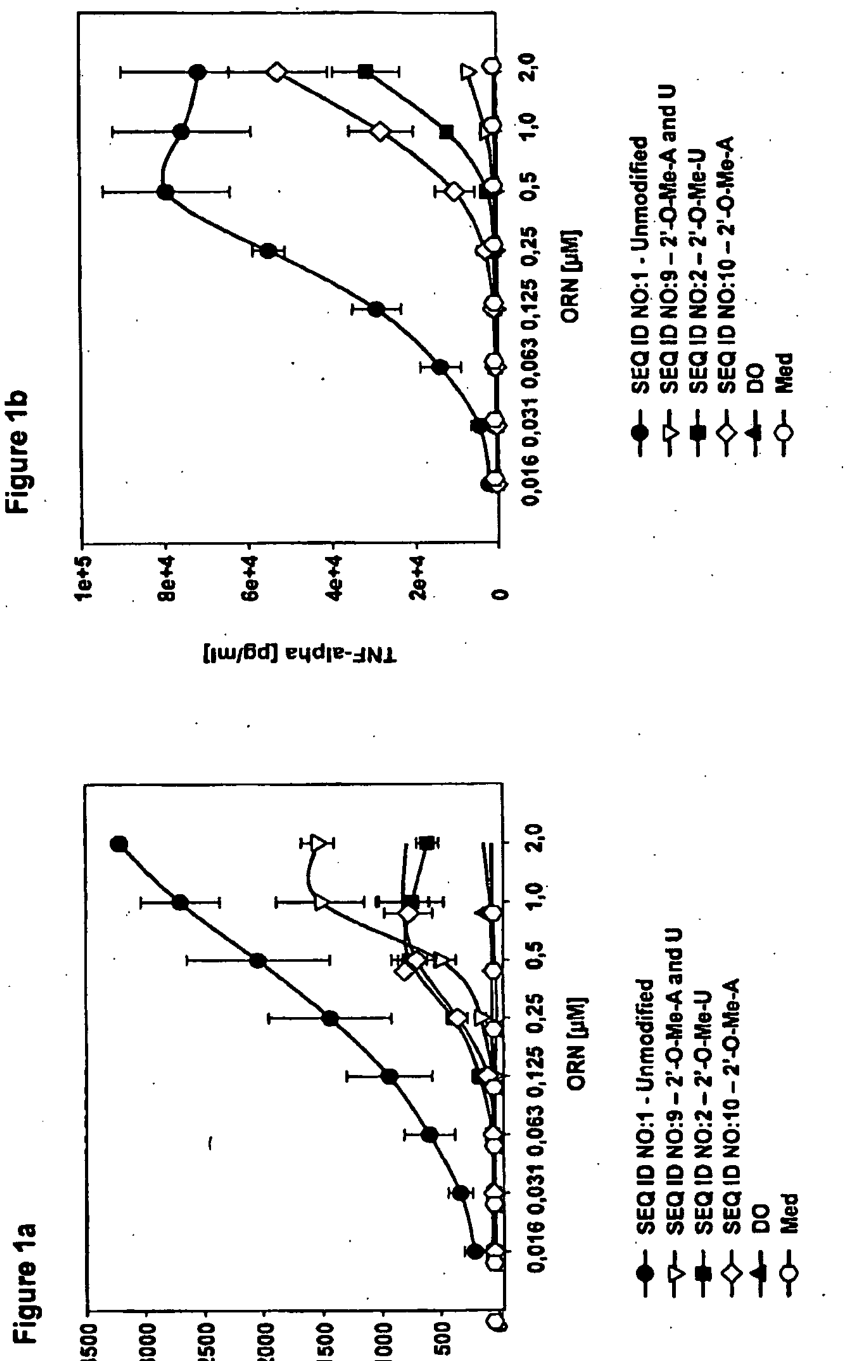


Figure 1a
IFN-alpha [pg/ml]
3500
3000
2500
2000
1500
1000
500
0
0,016 0,031 0,063 0,125 0,25 0,5 1,0 2,0
ORN [µM]
SEQ ID NO:1 - Unmodified
SEQ ID NO:9 – 2'-O-Me-A and U
SEQ ID NO:2 – 2'-O-Me-U
SEQ ID NO:10 – 2'-O-Me-A
DO
Med
Figure 1b
TNF-alpha [pg/ml]
1e+5
8e+4
6e+4
4e+4
2e+4
0
0,016 0,031 0,063 0,125 0,25 0,5 1,0 2,0
ORN [µM]
SEQ ID NO:1 - Unmodified
SEQ ID NO:9 – 2'-O-Me-A and U
SEQ ID NO:2 – 2'-O-Me-U
SEQ ID NO:10 – 2'-O-Me-A
DO
Med

Figure 1

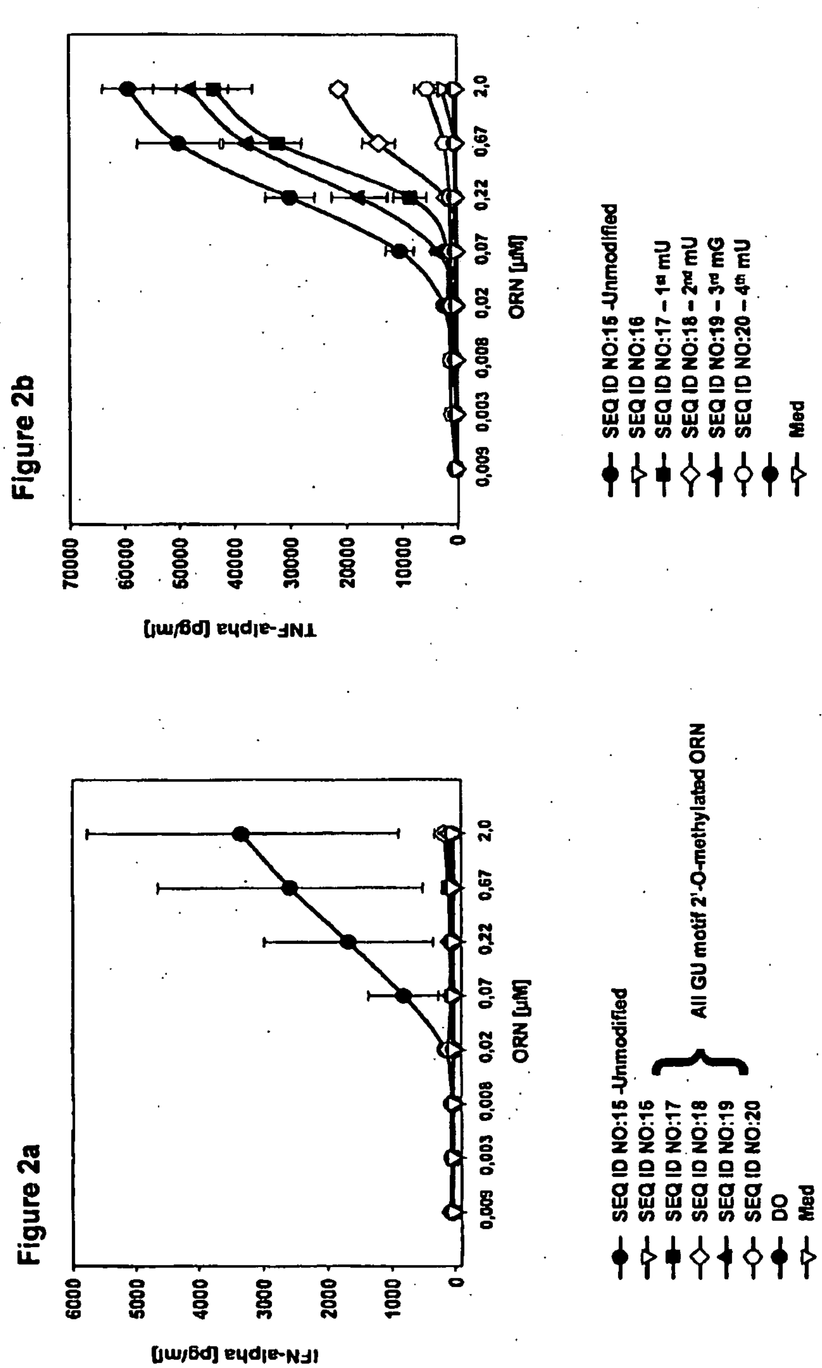


Figure 2a
IFN-alpha [pg/ml]
6000
5000
4000
3000
2000
1000
0
0,009 0,003 0,008 0,02 0,07 0,22 0,67 2,0
ORN [µM]
SEQ ID NO:15 -Unmodified
SEQ ID NO:16
SEQ ID NO:17
SEQ ID NO:18
SEQ ID NO:19
SEQ ID NO:20
DO
Med
All GU motif 2'-O-methylated ORN
Figure 2b
TNF-alpha [pg/ml]
70000
60000
50000
40000
30000
20000
10000
0
0,009 0,003 0,008 0,02 0,07 0,22 0,67 2,0
ORN [µM]
SEQ ID NO:15 -Unmodified
SEQ ID NO:16
SEQ ID NO:17 – 1st mU
SEQ ID NO:18 – 2nd mU
SEQ ID NO:19 – 3rd mG
SEQ ID NO:20 – 4th mU
Med

Figure 2

Figure 3a

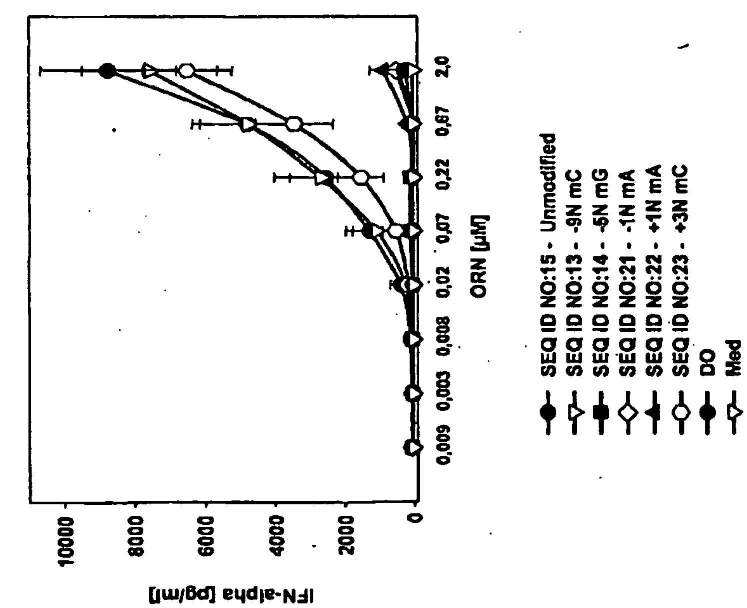


Figure 3b

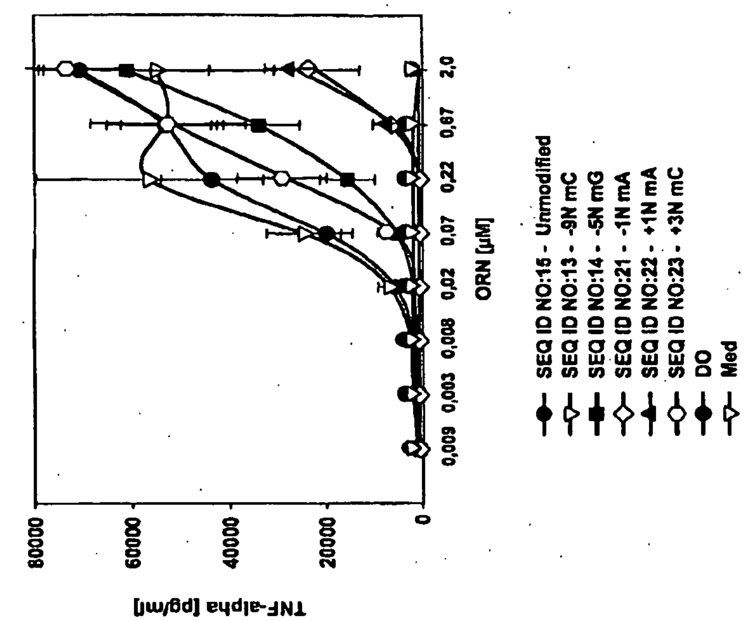


Figure 3

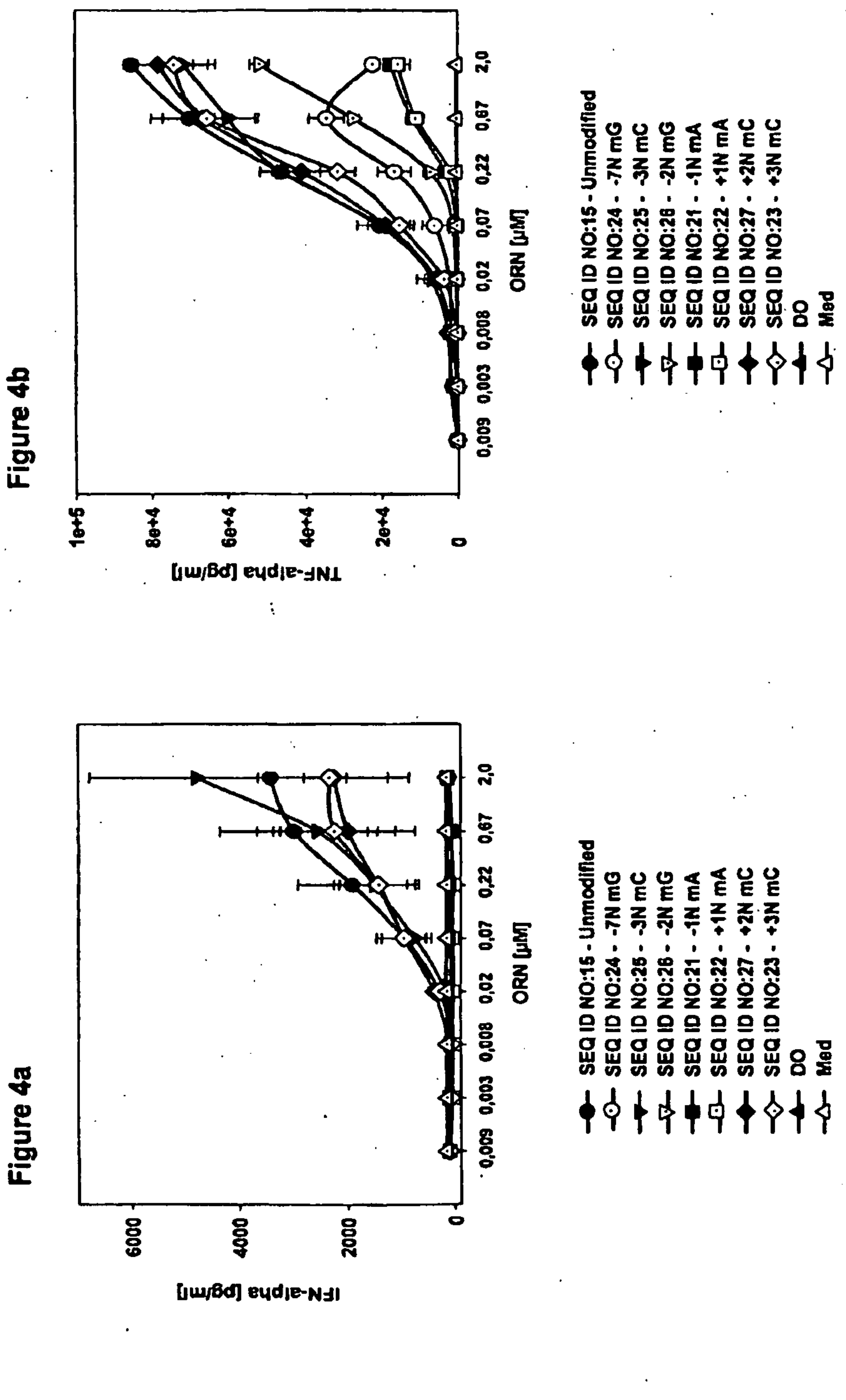


Figure 4a
IFN-alpha [pg/ml]
6000
4000
2000
0
0,009 0,003 0,008 0,02 0,07 0,22 0,67 2,0
ORN [µM]
SEQ ID NO:15 - Unmodified
SEQ ID NO:24 - -7N mG
SEQ ID NO:25 - -3N mC
SEQ ID NO:26 - -2N mG
SEQ ID NO:21 - -1N mA
SEQ ID NO:22 - +1N mA
SEQ ID NO:27 - +2N mC
SEQ ID NO:23 - +3N mC
DO
Med
Figure 4b
TNF-alpha [pg/ml]
1e+5
8e+4
6e+4
4e+4
2e+4
0
0,009 0,003 0,008 0,02 0,07 0,22 0,67 2,0
ORN [µM]
SEQ ID NO:15 - Unmodified
SEQ ID NO:24 - -7N mG
SEQ ID NO:25 - -3N mC
SEQ ID NO:26 - -2N mG
SEQ ID NO:21 - -1N mA
SEQ ID NO:22 - +1N mA
SEQ ID NO:27 - +2N mC
SEQ ID NO:23 - +3N mC
DO
Med

Figure 4

Figure 5b

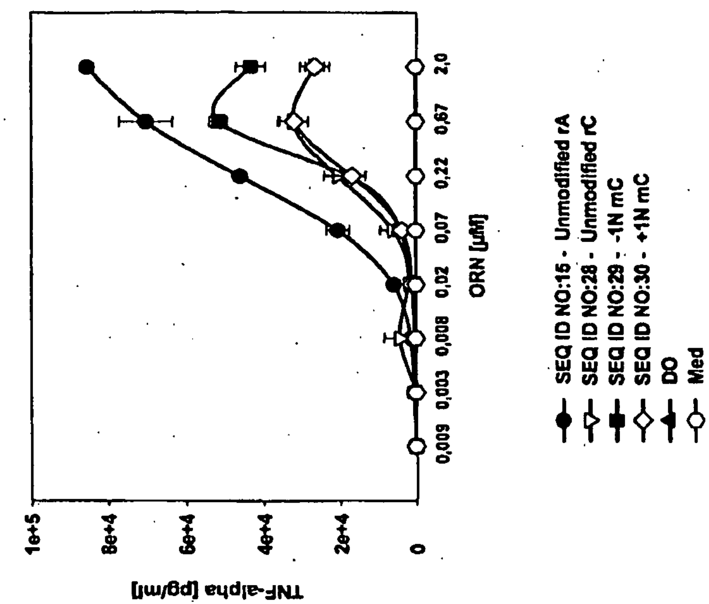


Figure 5a

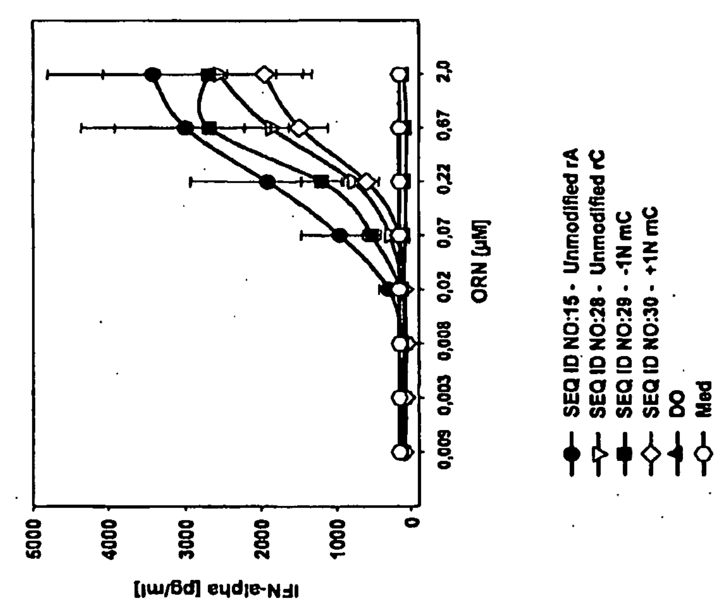


Figure 5

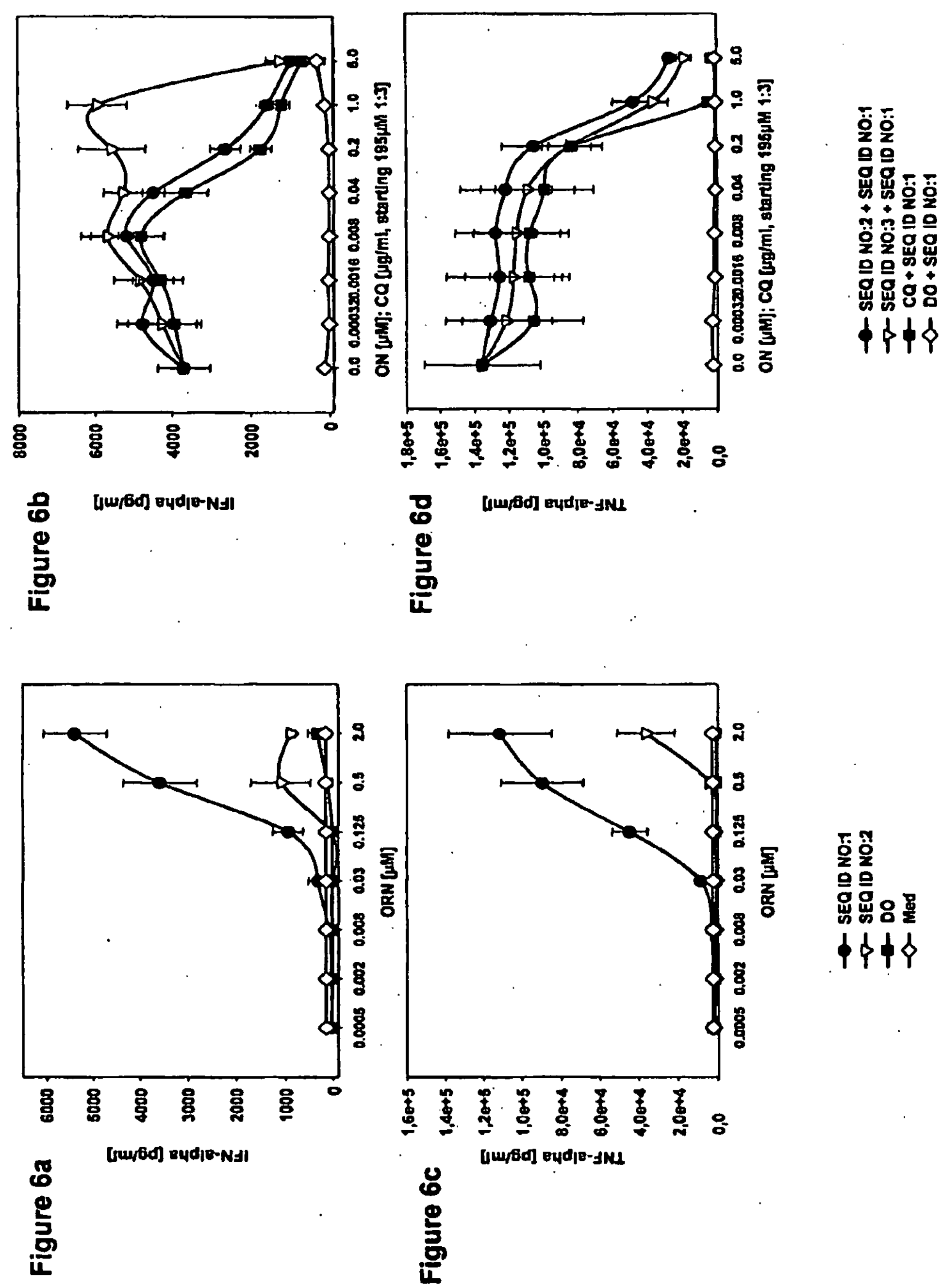


Figure 6a
IFN-alpha [pg/ml]
ORN [µM]
Figure 6b
IFN-alpha [pg/ml]
ON [µM]; CQ [µg/ml, starting 195µM 1:3]
Figure 6c
TNF-alpha [pg/ml]
ORN [µM]
Figure 6d
TNF-alpha [pg/ml]
ON [µM]; CQ [µg/ml, starting 195µM 1:3]
SEQ ID NO:1
SEQ ID NO:2
DO
Med
SEQ ID NO:2 + SEQ ID NO:1
SEQ ID NO:3 + SEQ ID NO:1
CQ + SEQ ID NO:1
DO + SEQ ID NO:1

Figure 6

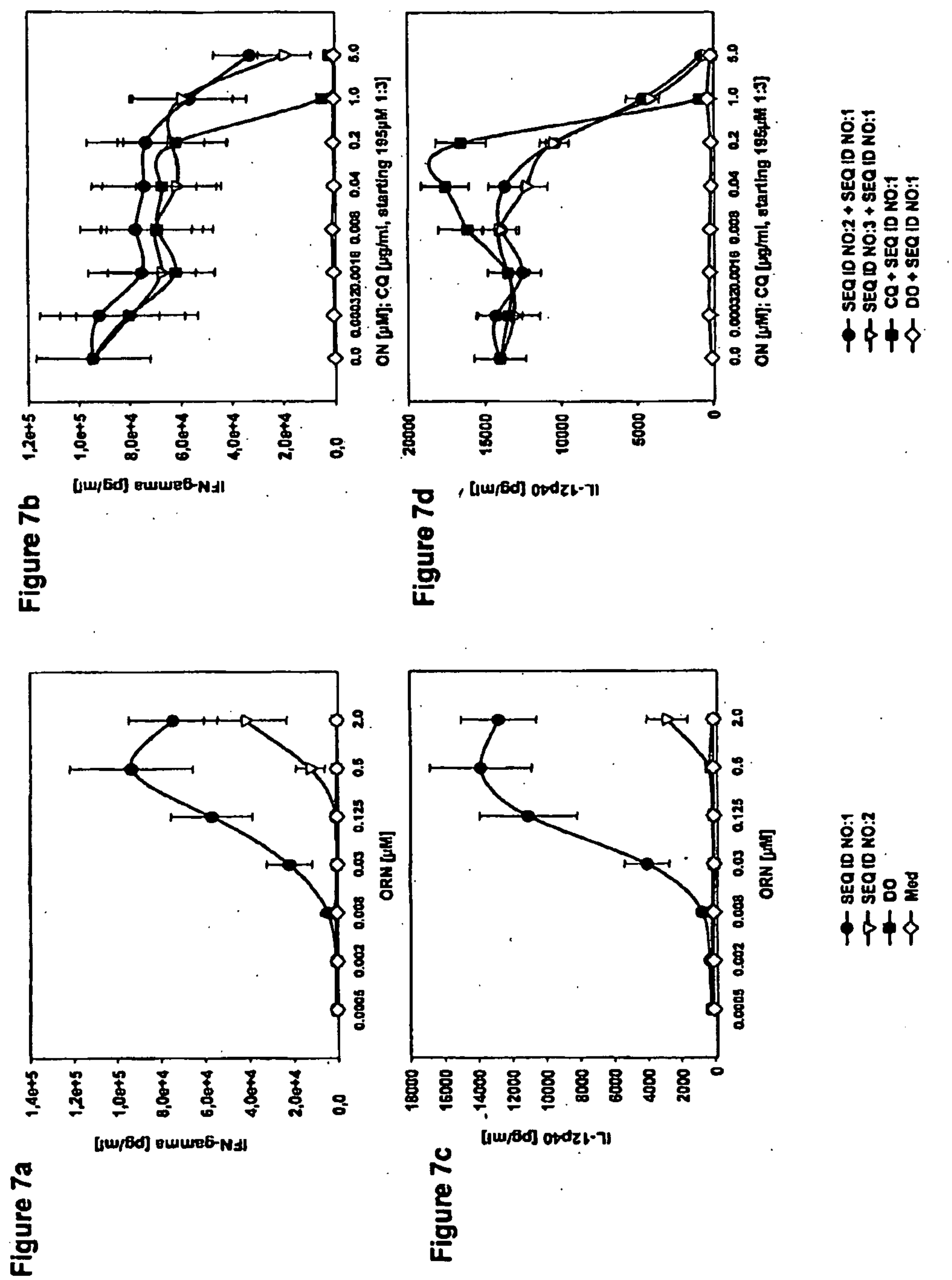


Figure 7a
IFN-gamma [pg/ml]
ORN [µM]
Figure 7b
IFN-gamma [pg/ml]
ON [µM]; CQ [µg/ml, starting 195µM 1:3]
Figure 7c
IL-12p40 [pg/ml]
ORN [µM]
Figure 7d
IL-12p40 [pg/ml]
ON [µM]; CQ [µg/ml, starting 195µM 1:3]
SEQ ID NO:1
SEQ ID NO:2
DO
Med
SEQ ID NO:2 + SEQ ID NO:1
SEQ ID NO:3 + SEQ ID NO:1
CQ + SEQ ID NO:1
DO + SEQ ID NO:1

Figure 7

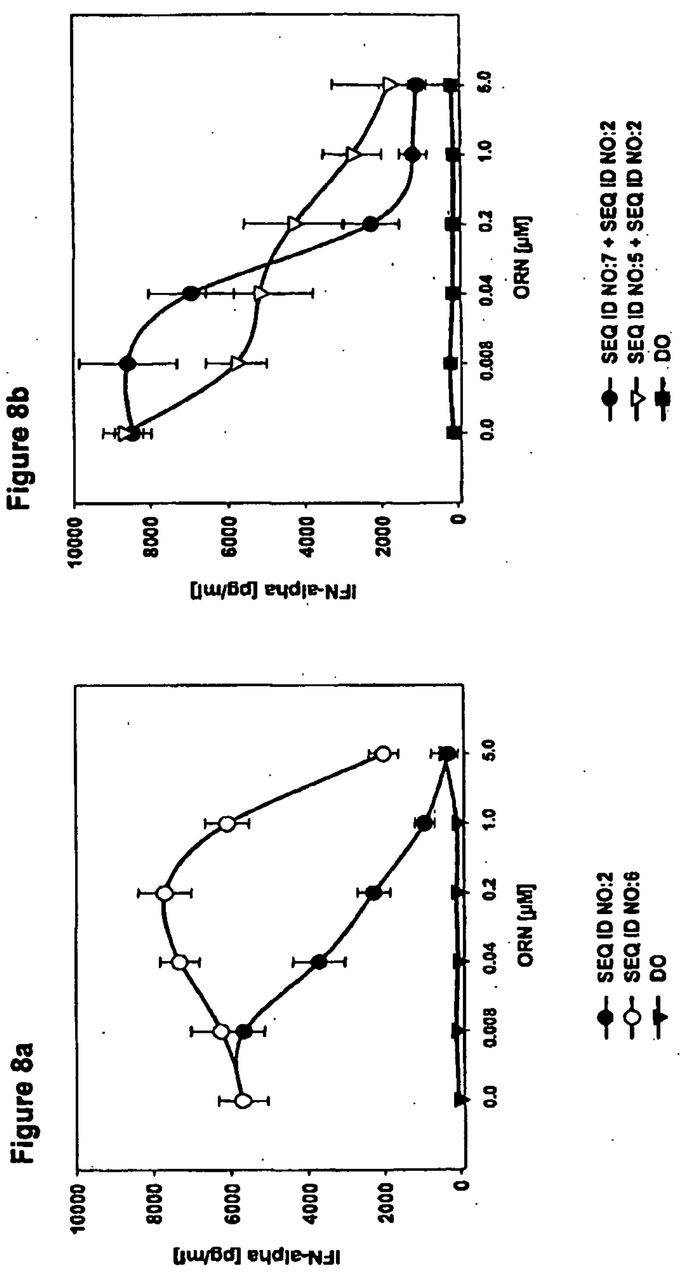


Figure 8a
IFN-alpha [pg/ml]
10000
8000
6000
4000
2000
0
0.0
0.008
0.04
0.2
1.0
5.0
ORN [µM]
SEQ ID NO:2
SEQ ID NO:6
DO
Figure 8b
IFN-alpha [pg/ml]
10000
8000
6000
4000
2000
0
0.0
0.008
0.04
0.2
1.0
5.0
ORN [µM]
SEQ ID NO:7 + SEQ ID NO:2
SEQ ID NO:5 + SEQ ID NO:2
DO

Figure 8

Figure 9a

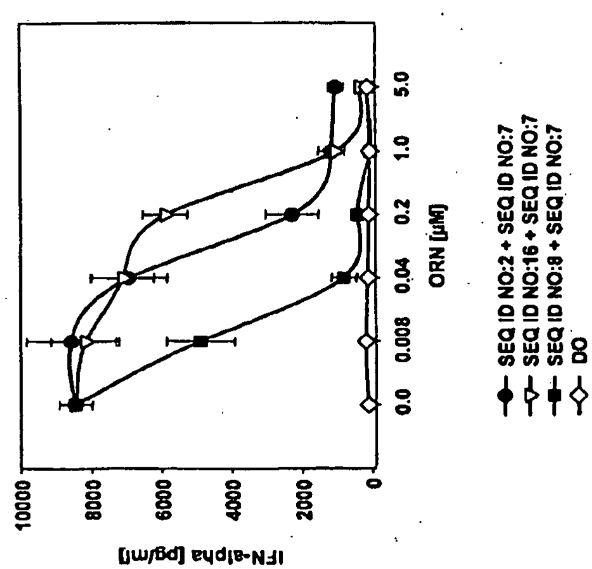


Figure 9b

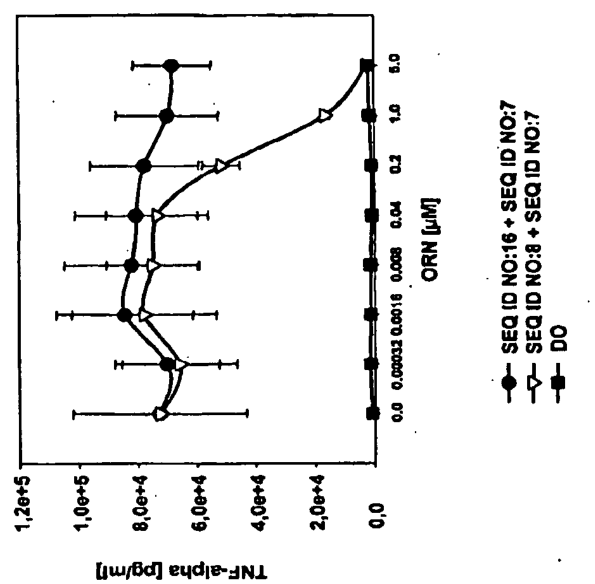


Figure 9

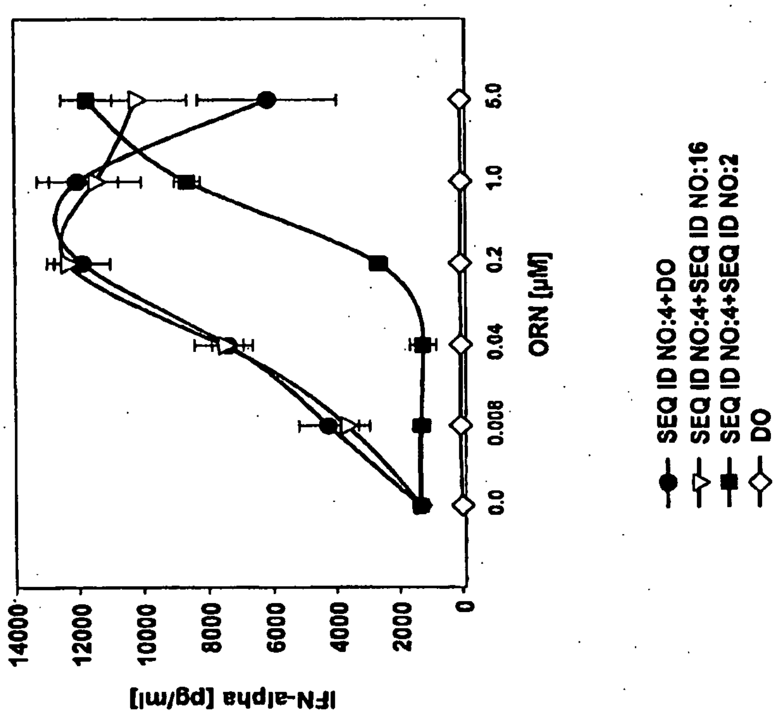

IFN-alpha [pg/ml]
14000
12000
10000
8000
6000
4000
2000
0
0.0
0.008
0.04
0.2
1.0
5.0
ORN [µM]
SEQ ID NO:4+DO
SEQ ID NO:4+SEQ ID NO:16
SEQ ID NO:4+SEQ ID NO:2
DO

Figure 10

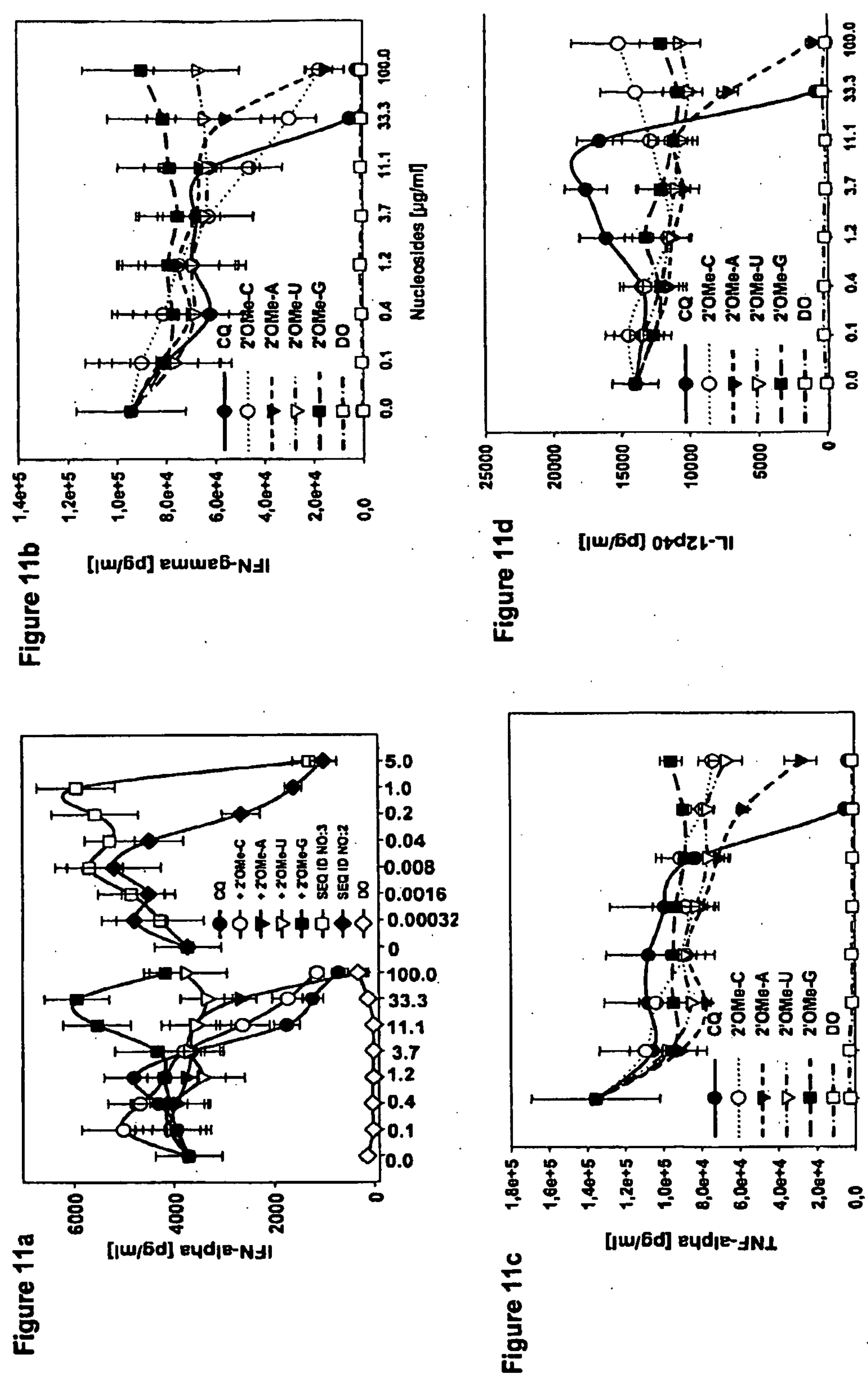


Figure 11a
IFN-alpha [pg/ml]
CQ
+ 2'OMe-C
+ 2'OMe-A
+ 2'OMe-U
+ 2'OMe-G
SEQ ID NO:3
SEQ ID NO:2
DO
Figure 11b
IFN-gamma [pg/ml]
Nucleosides [µg/ml]
CQ
2'OMe-C
2'OMe-A
2'OMe-U
2'OMe-G
DO
Figure 11c
TNF-alpha [pg/ml]
Nucleosides [µg/ml]
Figure 11d
IL-12p40 [pg/ml]
Nucleosides [µg/ml]

Figure 11

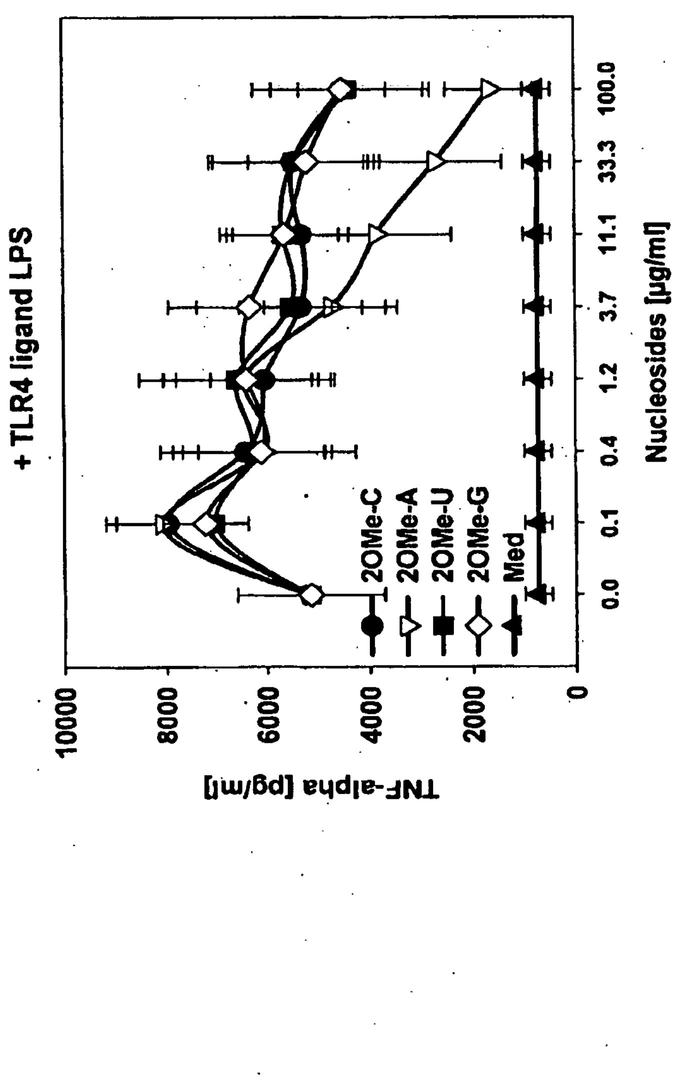

+ TLR4 ligand LPS
TNF-alpha [pg/ml]
10000
8000
6000
4000
2000
0
0.0
0.1
0.4
1.2
3.7
11.1
33.3
100.0
Nucleosides [µg/ml]
2OMe-C
2OMe-A
2OMe-U
2OMe-G
Med

Figure 12

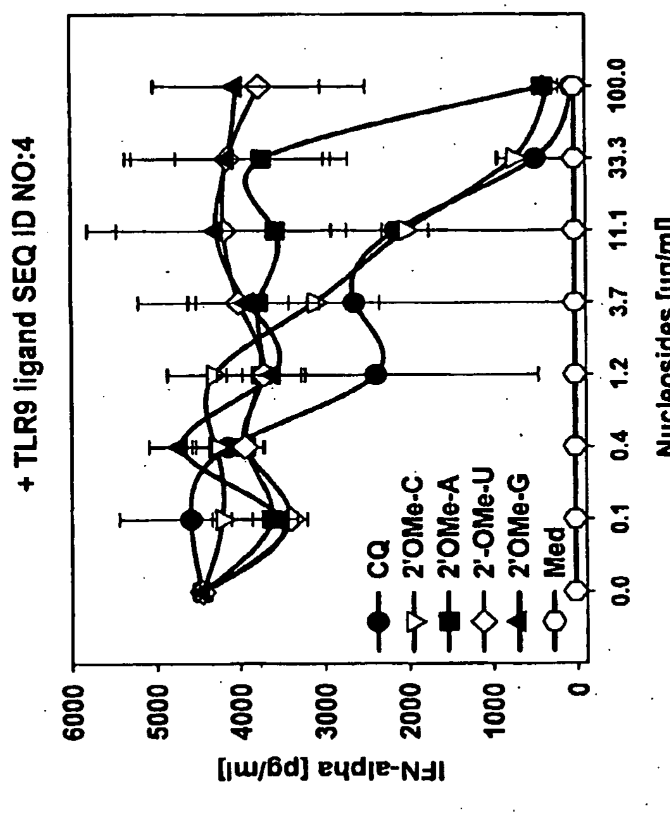

+ TLR9 ligand SEQ ID NO:4
IFN-alpha [pg/ml]
6000
5000
4000
3000
2000
1000
0
CQ
2'OMe-C
2'OMe-A
2'-OMe-U
2'OMe-G
Med
0.0
0.1
0.4
1.2
3.7
11.1
33.3
100.0
Nucleosides [µg/ml]

Figure 13

Figure 14a

Figure 14b

Figure 14c

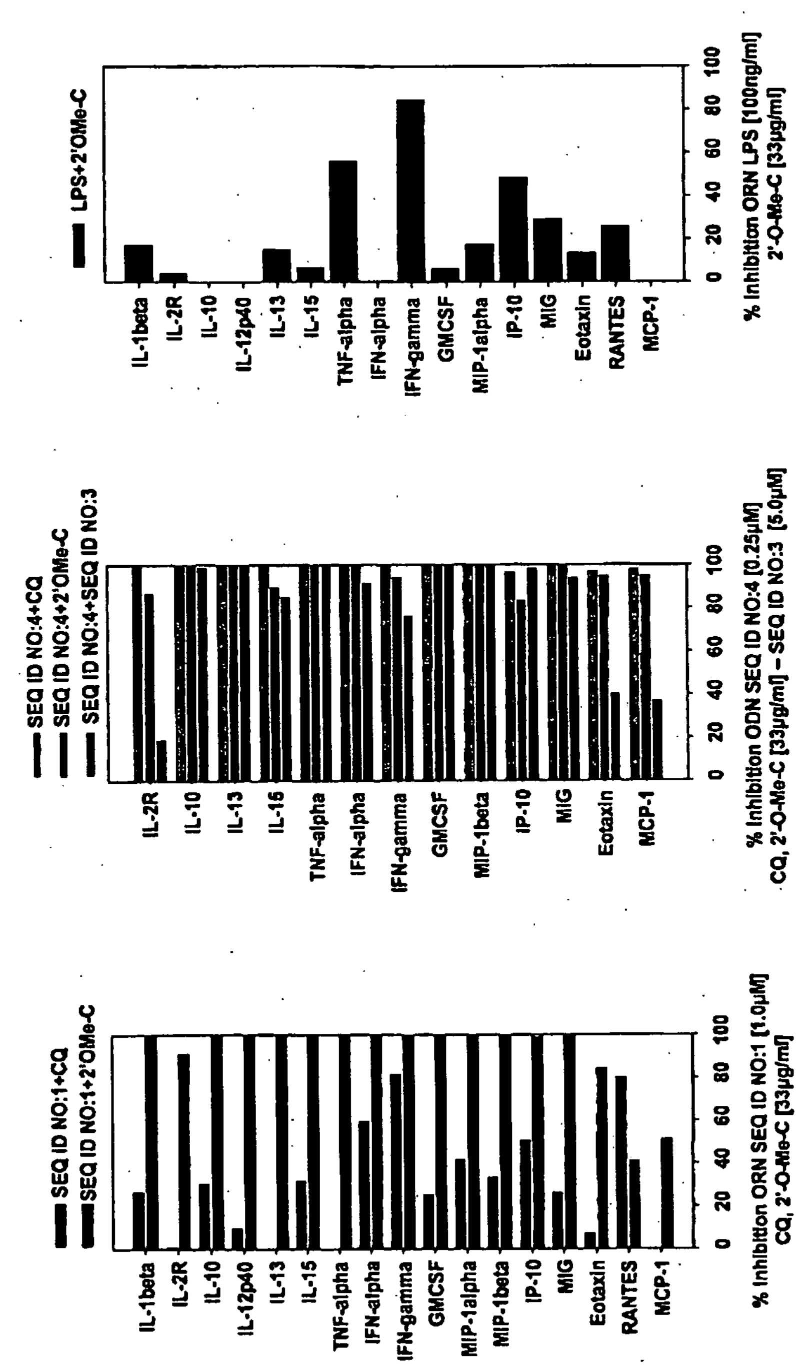


Figure 14

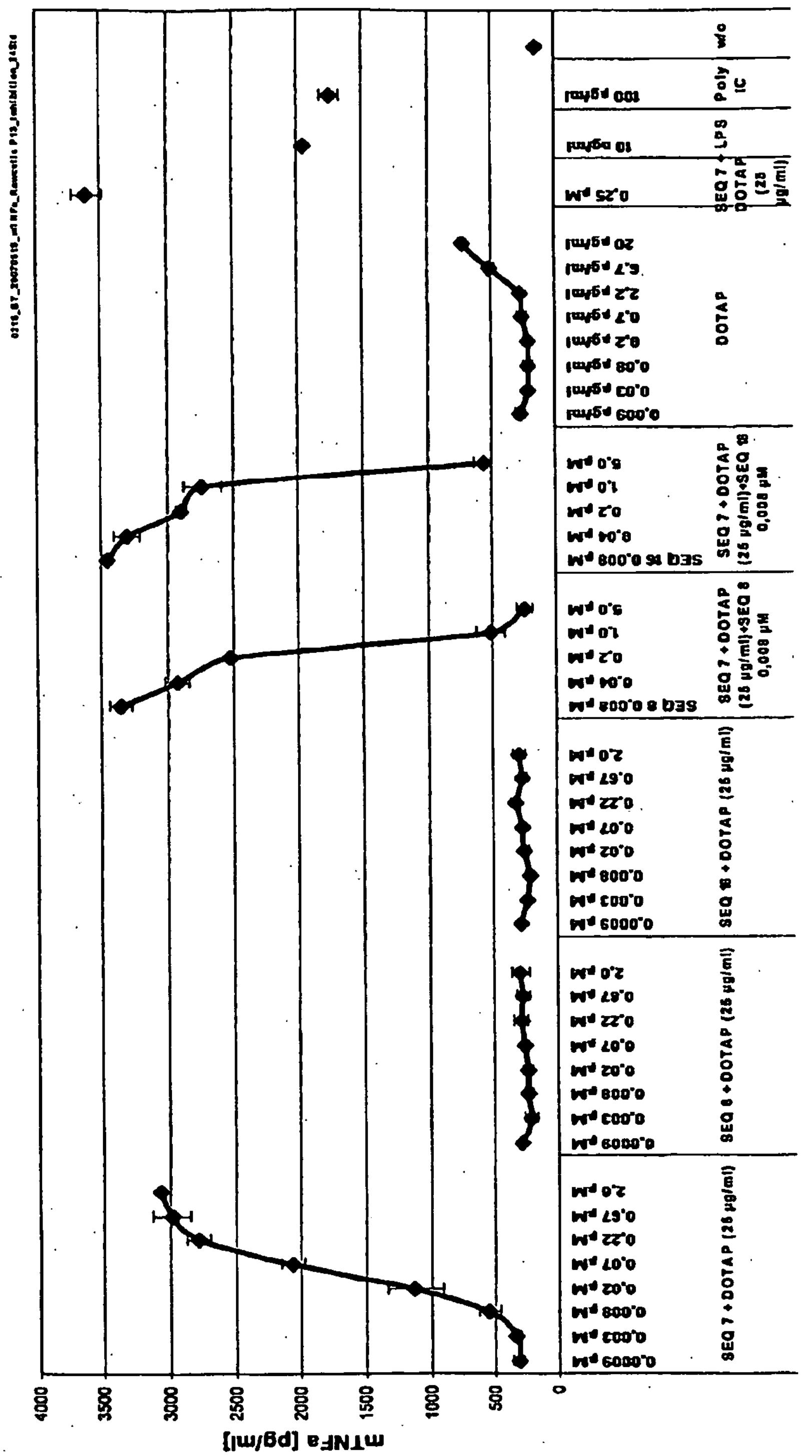

mTNFa [pg/ml]
0
500
1000
1500
2000
2500
3000
3500
4000
SEQ 7 + DOTAP (25 µg/ml)
SEQ 8 + DOTAP (25 µg/ml)
SEQ 18 + DOTAP (25 µg/ml)
SEQ 7 + DOTAP (25 µg/ml)+SEQ 8 0,008 µM
SEQ 7 + DOTAP (25 µg/ml)+SEQ 18 0,008 µM
DOTAP
SEQ 7 + DOTAP (25 µg/ml)
LPS
Poly IC
w/o
ODN_ORN [µM]

Figure 15

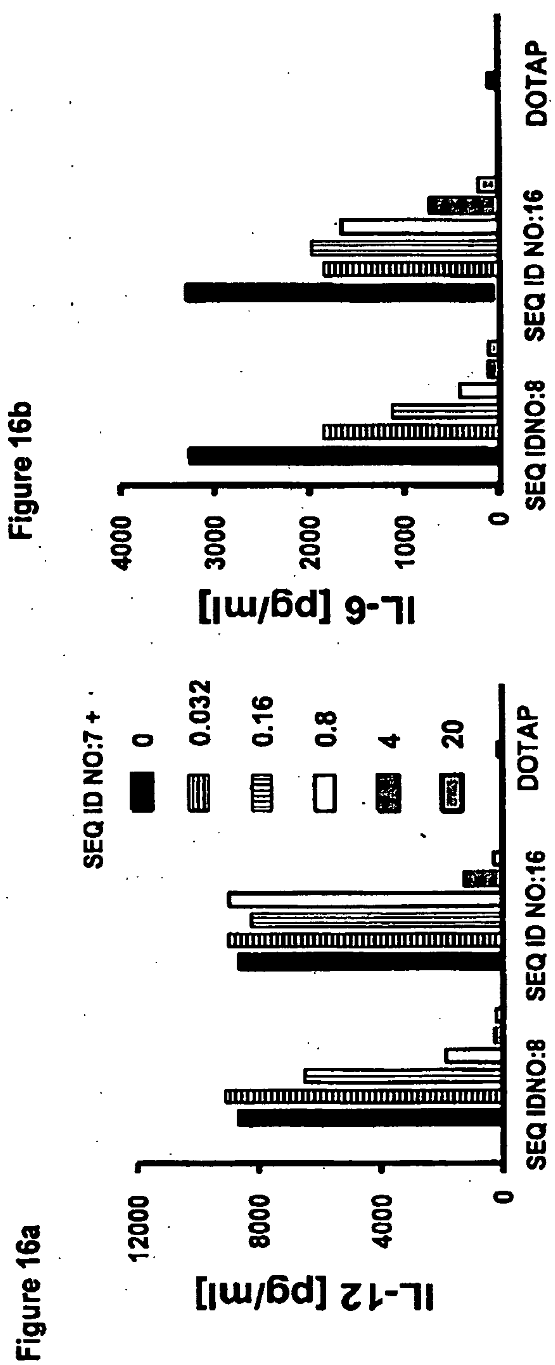


Figure 16a
IL-12 [pg/ml]
12000
8000
4000
0
SEQ IDNO:8
SEQ ID NO:16
DOTAP
SEQ ID NO:7 +
0
0.032
0.16
0.8
4
20
Figure 16b
IL-6 [pg/ml]
4000
3000
2000
1000
0
SEQ IDNO:8
SEQ ID NO:16
DOTAP

Figure 16